



US008410333B2

(12) **United States Patent**
Buelow et al.

(10) **Patent No.:** **US 8,410,333 B2**
(45) **Date of Patent:** **Apr. 2, 2013**

(54) **HUMANIZED IMMUNOGLOBULIN LOCI**

(75) Inventors: **Roland Buelow**, Palo Alto, CA (US);
Wim van Schooten, Sunnyvale, CA
(US); **Josef Platzer**, Geretsried (DE)

(73) Assignee: **Therapeutic Human Polyclonals, Inc.**,
Mountain View, CA (US)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

(21) Appl. No.: **12/511,188**

(22) Filed: **Jul. 29, 2009**

(65) **Prior Publication Data**

US 2010/0205682 A1 Aug. 12, 2010

Related U.S. Application Data

(62) Division of application No. 10/893,483, filed on Jul.
15, 2004, now Pat. No. 7,585,668.

(60) Provisional application No. 60/487,733, filed on Jul.
15, 2003.

(51) **Int. Cl.**
A01K 67/027 (2006.01)

(52) **U.S. Cl.** **800/14; 800/13**

(58) **Field of Classification Search** **800/14,**
800/13

See application file for complete search history.

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

EP	0 491 057	7/1991
EP	0 583 980	8/1993
WO	WO 92/03918	3/1992
WO	WO 97/16537	9/1997
WO	WO 00/46251	8/2000
WO	WO 00/75300	12/2000
WO	WO 01/19394	3/2001
WO	WO 02/12437	* 2/2002
WO	WO 2004/003157	1/2004

OTHER PUBLICATIONS

Butler, "Immunoglobulin Diversity, B-cell and Antibody Repertoire
Development in Large Farm Animals" Rev. Sci. Tech. Off. int. Epiz.
17(1):43-70 (1998).

Etches et al., "Strategies for the Production of Transgenic Chickens"
Methods in Molecular Biology 62:433-450.

Hole et al., "Identification of Enhancer Sequences 3' of the Rabbit
Ig_RL Chain Loci" The Journal of Immunology 146(12):4377-4384
(1991).

Lauster et al., "Promotor, Enhancer and Silencer Elements Regulate
Rearrangement of an Immunoglobulin Transgene" EMBO Journal
12(12):4615-4623 (1993).

Lee et al., "A Highly Efficient *Escherichia coli*-Based Chromosome
Engineering System Adapted for Recombination Targeting and
Subcloning of BAC DNA" Genomics 73:56-65 (2001).

Muyrers et al., "Rapid Modification of Bacterial Artificial Chromo-
somes by ET-recombination" Nucleic Acids Research 27(6):1555-
15557 (1997).

Reynaud et al., "Somatic Hyperconversion Diversifies the Single V_H
Gene of the Chicken with a High Incidence in the D Region" Cell
59:171-183 (1989).

Volgina et al., "A Single 3'α_{1,2} Enhancer in the rabbit IgH Locus"
Journal of Immunology 165:6400-6405 (2000).

Yu et al., "An Efficient Recombination System for Chromosome
Engineering in *Escherichia coli*" PNAS, vol. 97. No. 11. pp. 5987-
5983 (2000).

Zhang et al., "Risk of Tuberculosis Infection and Tuberculous Men-
ingitis after Discontinuation of *Bacillus Calmette-Guerin* in Beijing"
Am J Respir Crit Care Med 162:1314-1317 (2000).

Database EMBL Jul. 16, 1998, "rabbit Ig Germline CL42. Vh25 and
CL. 42Vh34 Genes, VHA3 Allotype, 5' end" XP002393777 retrieved
from FBI Database Accession No. M12180.

Dufour et al., "The Sheep Ig Variable Region Repertoire Consists of
Single V_H Family" Journal of Immunology, 156:2163-2170 (1996).

Genabnk. A Y495826, Platzer. J., Direct Submission; Nov. 2003.
THP GmbH, Am Neuland 3, Bernried 82347 Germany.

Genbank. A Y495827, Platzer, J., Direct Submission: Nov. 2003. THP
GmbH, Am Neuland 3, Bernried 82347 Germany.

Genbank, A Y495828. Platzer. J.. "Sequence Analysis of 0.4
Megabases of the rabbit Germline Immunoglobulin Kappal Light
Chain Locus" Direct Submission: Nov. 2003. THP GmbH, Am
Neuland 3, Bernried 82347 Germany.

Hesse et al., "V(D)J Recombination: A Functional Definition of the
Joining Signals" Genes and Development, Cold Spring Harbor Labo-
ratory Press, Plainview, NY, US, vol. 3. No. 7, (1989), pp. 1053-1061
XP009043633 ISSN: 0890-9369.

Mansikka et al., "Bursectomy of Chicken Embryos at 60 Hours of
Incubation Leads to an Oligoclonal B Cell Compartment and
Restricted Ig Diversity" Journal of Immunology, 145(11):3601-3609
(1990).

Muyrers et al., "Techniques: Recombinogenic Engineering-New
Options for Cloning and Manipulating DNA" Trends in Biochemical
Sciences, 26(5):325-331 (2001).

Pain et al., "Chicken Embryonic Stem Cells and Transgenic Strate-
gies" Cell Tissues, 165:212-219 (1999).

Reynaud et al., "Hypermutation Generating the Sheep
Immunoglobulin Repertoire is an Antigen-Independent Process"
Cell, 80:115-125 (1995).

Sang, "Transgenic Chicken-Methods and Potential Applications"
Tibtech, 12:415-420 (1994).

Sherman et al., "Transposition of the *Drosophila* Element Mariner
into the Chicken Germ Line" Nature Biotechnology, 16:1050-1053
(1998).

Bruggermann, et al., "A Repertoire of monoclonal antibodies with
human heavy chains from transgenic mice", Proceedings of the
national academy of sciences, vol. 86, No. 17, pp. 6709-6713, (1989).

Bruggermann, et al., "Strategies for expressing human antibody rep-
ertoires in transgenic mice", Immunology. vol. 17. No. 8, pp. 391-
397, (1996).

(Continued)

Primary Examiner — Anne Marie S Wehbe

(74) *Attorney, Agent, or Firm* — Ginger R. Dreger; Arnold &
Porter LLP

(57) **ABSTRACT**

The present invention concerns methods and means to pro-
duce humanized antibodies from transgenic non-human ani-
mals. The invention specifically relates to novel immunoglo-
bulin heavy and light chain constructs, recombination and
transgenic vectors useful in making transgenic non-human
animals expressing humanized antibodies, transgenic ani-
mals, and humanized immunoglobulin preparations.

2 Claims, 49 Drawing Sheets

OTHER PUBLICATIONS

Jakobovitis, et al., "Analysis of homozygous mutant chimeric mice: deletion of the immunoglobulin heavy-chain region blocks b-cell development and antibody production", Proceedings of the national academy of sciences, vol. 90, No. 6, pp. 2551-2555, (1993).
Ros, at al., "Sequence analysis of 0.5 Mb of the rabbit germline immunoglobulin heavy chain locus", Gene: An international journal on genes and genomes, vol. 330, No. 14, pp. 49-59, (2004).

Database EMBL, Sep. 23, 2003, Acc. No. AY386697: <http://www.ebi.ac.uk/ena/data/view/AY386697>[Jun. 12, 2012] 21 pgs.
Knight, K.L., et al., "Generating the Antibody Repertoire in Rabbit", *Advances in Immunology*, vol. 56 (1994) pp. 179-218.
McCormack, Wayne T. et al., "Chicken Ig_L variable region gene conversion display pseudogene donor preference and 5' to 3' polarity", *Genes & Development* 4: (1990) pp. 548-558.

* cited by examiner

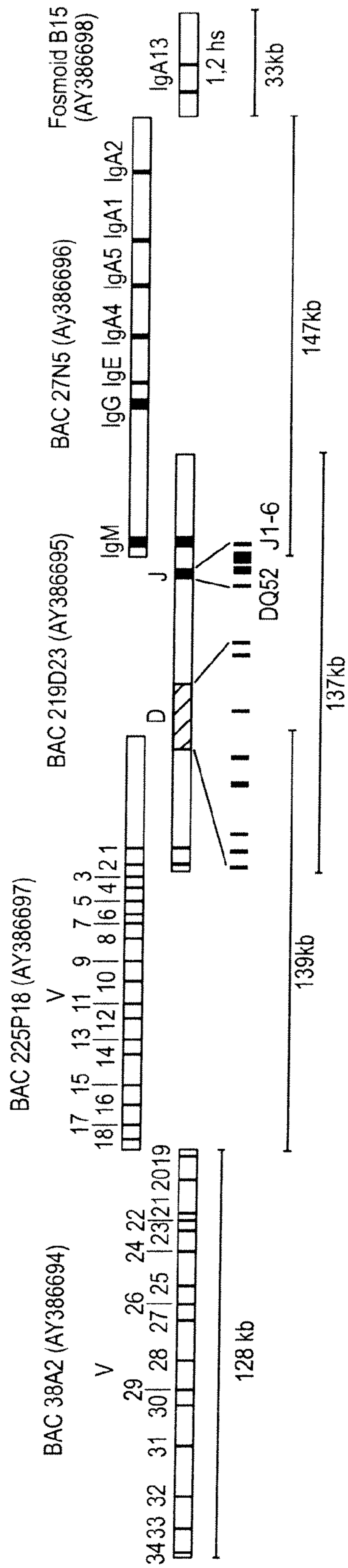


Figure 1

Figure 2

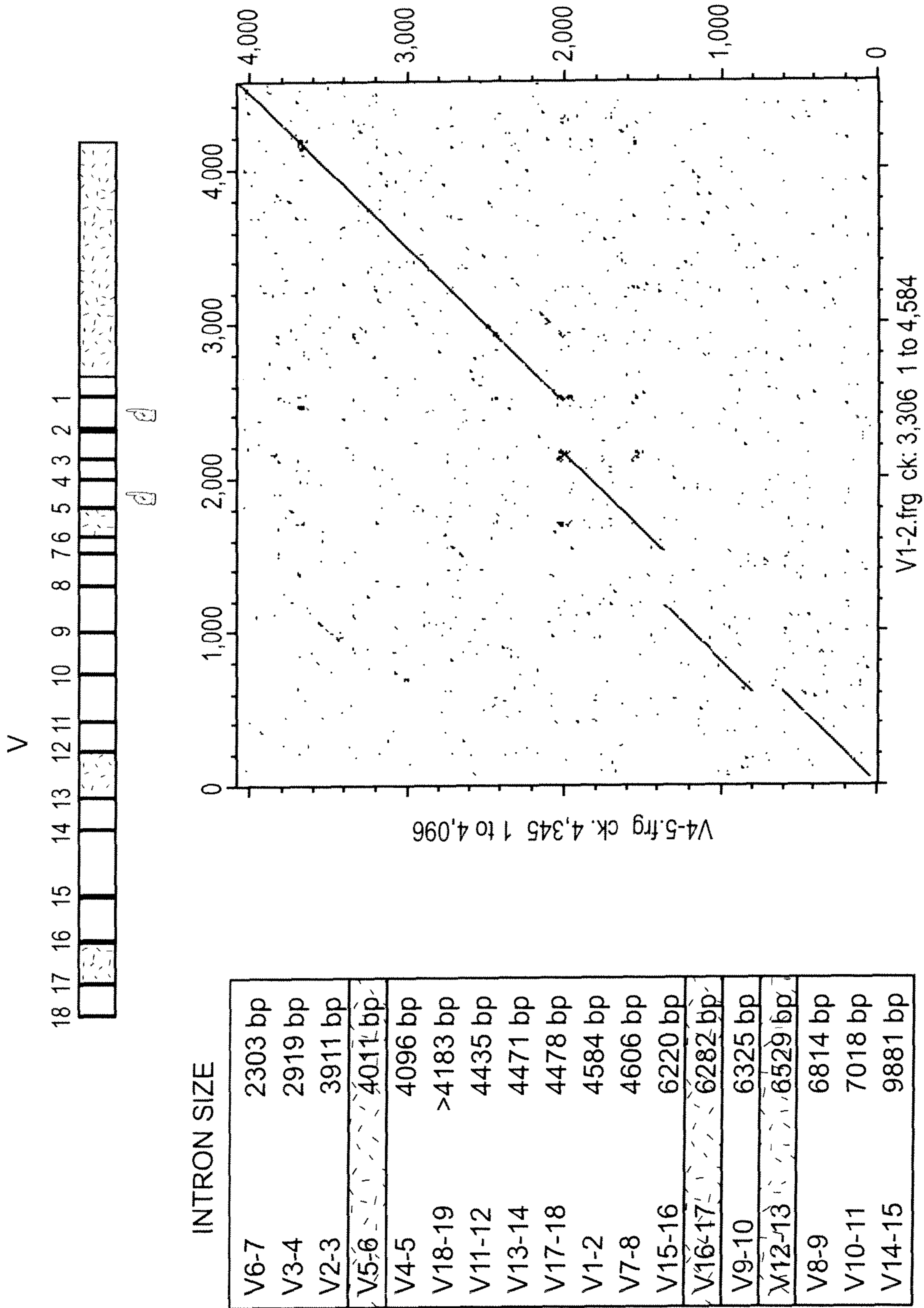
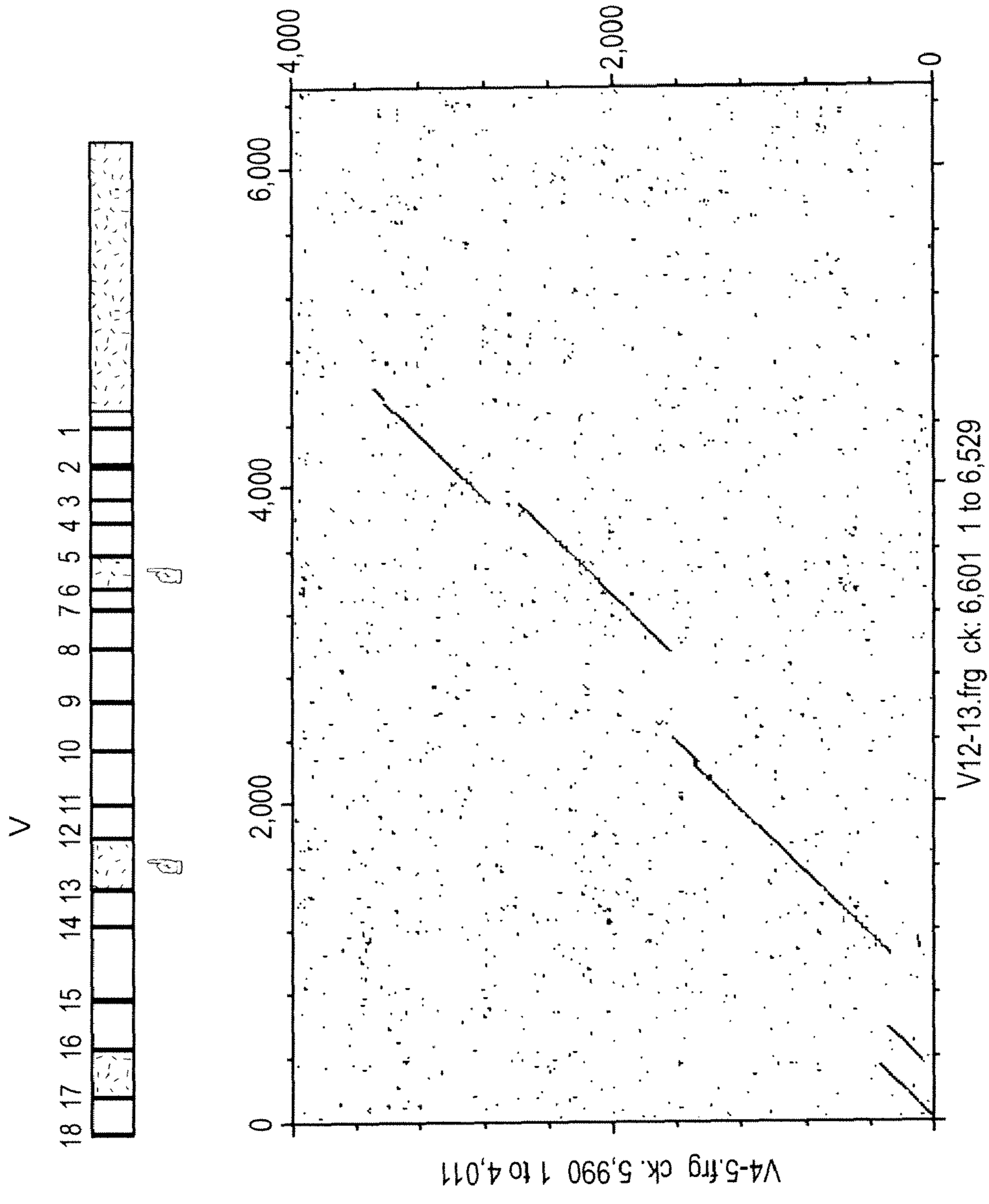


Figure 3



INTRON SIZE

V6-7	2303 bp
V3-4	2919 bp
V2-3	3911 bp
V5-6	4011 bp
V4-5	4096 bp
V18-19	>4183 bp
V11-12	4435 bp
V13-14	4471 bp
V17-18	4478 bp
V1-2	4584 bp
V7-8	4606 bp
V15-16	6220 bp
V16-17	6282 bp
V9-10	6325 bp
V12-13	6529 bp
V8-9	6814 bp
V10-11	7018 bp
V14-15	9881 bp

Figure 4

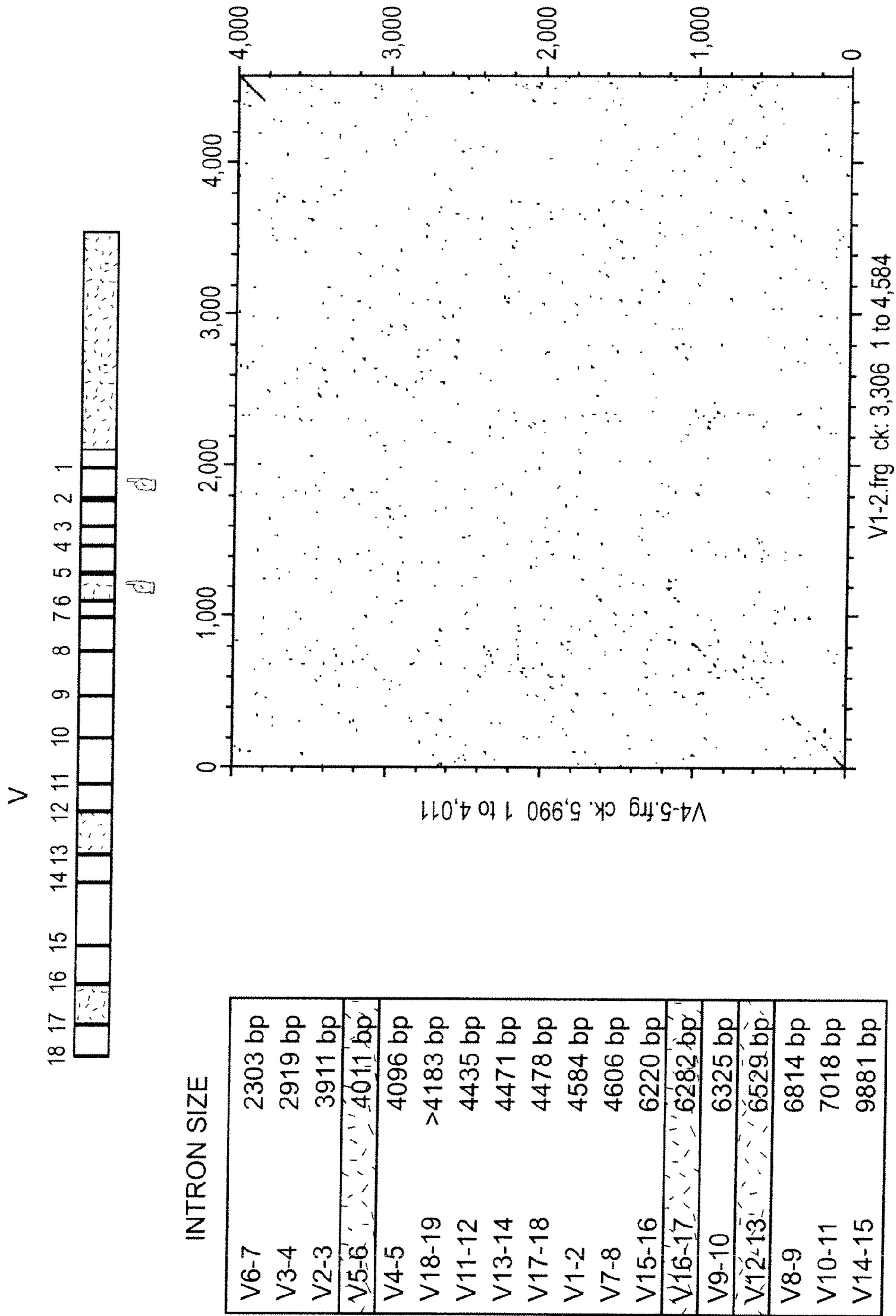


Figure 5

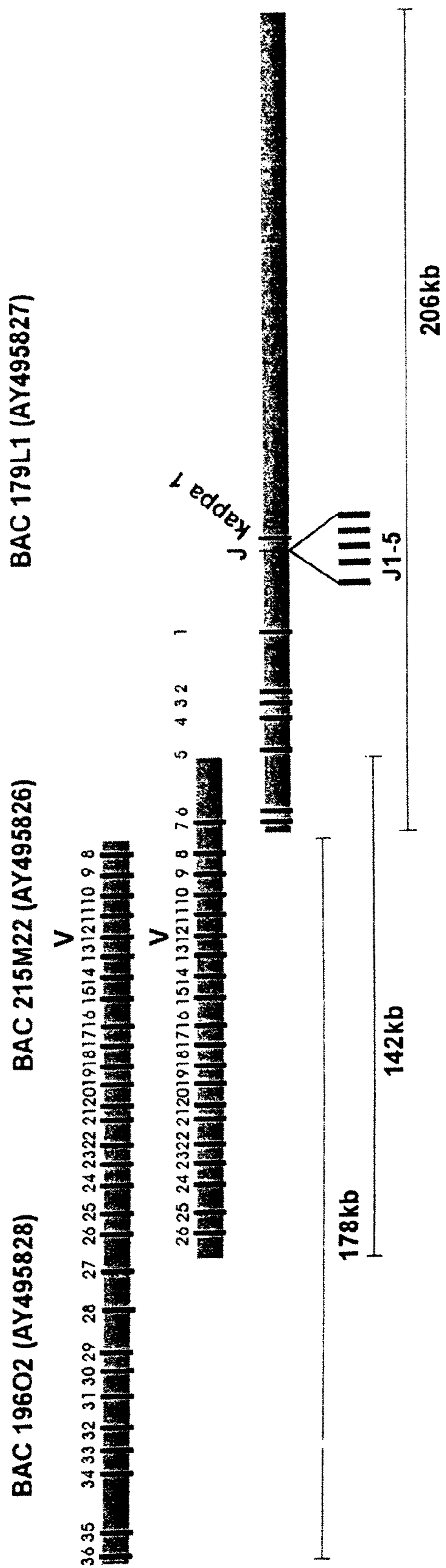


Figure 6

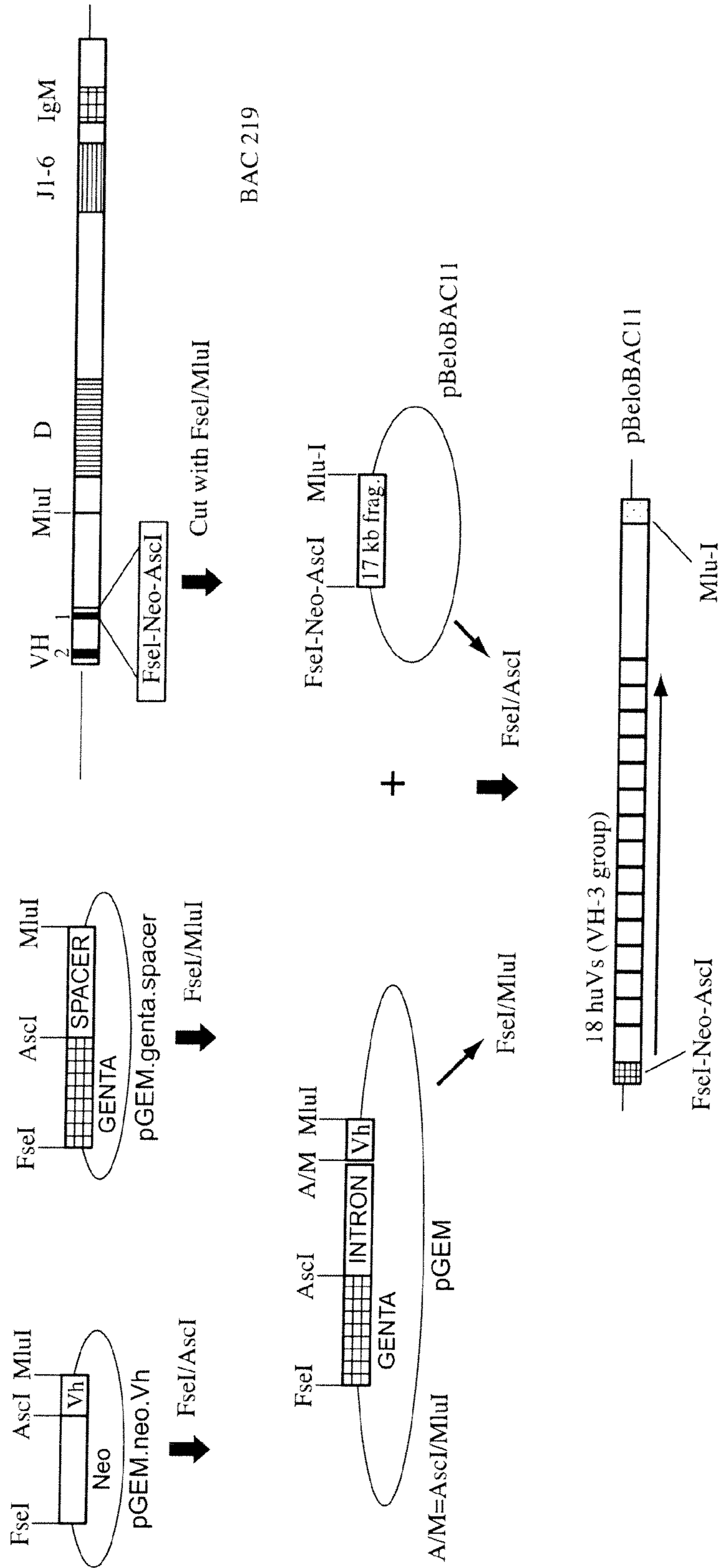
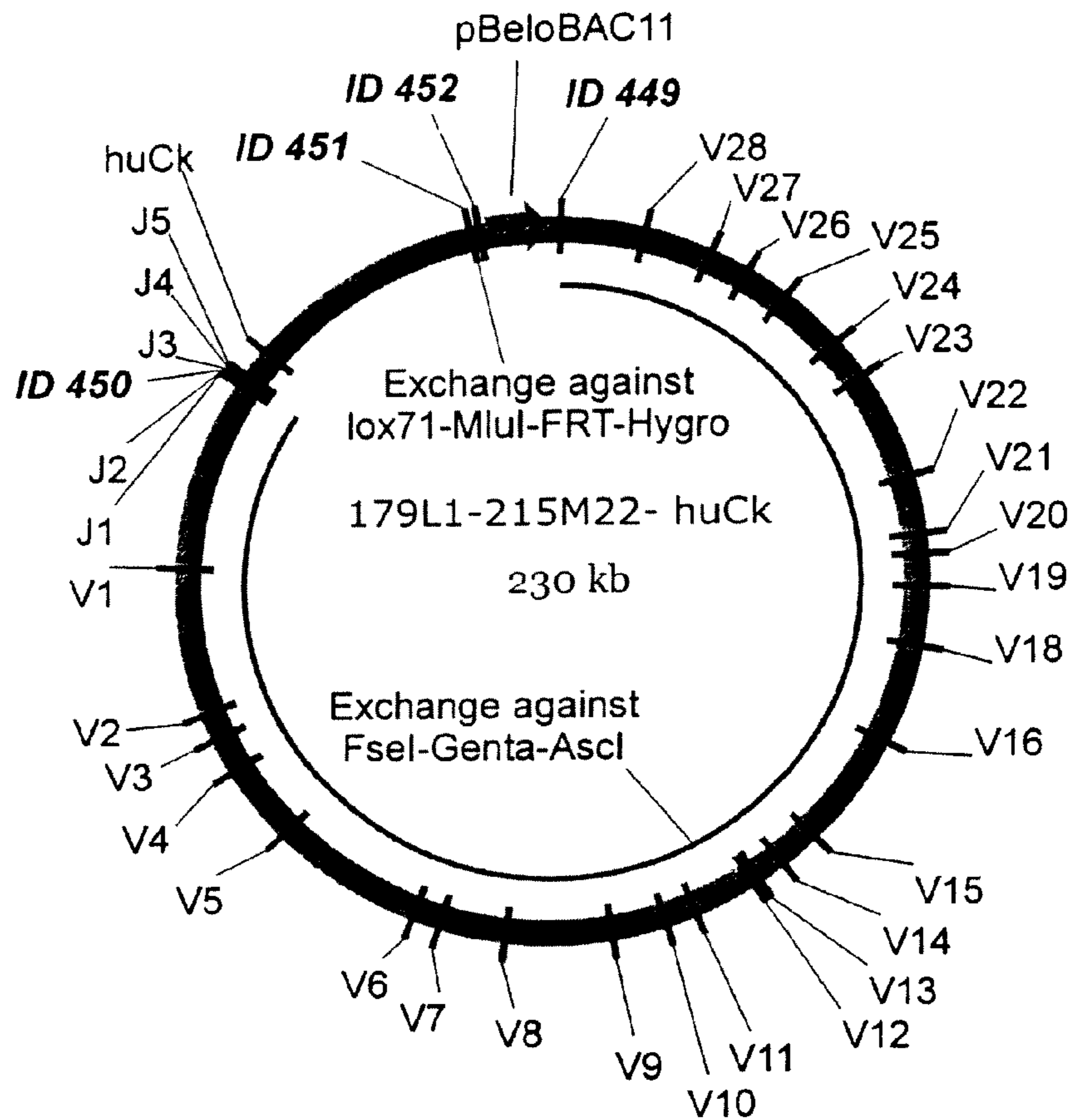


Figure 7

(a)



(b)

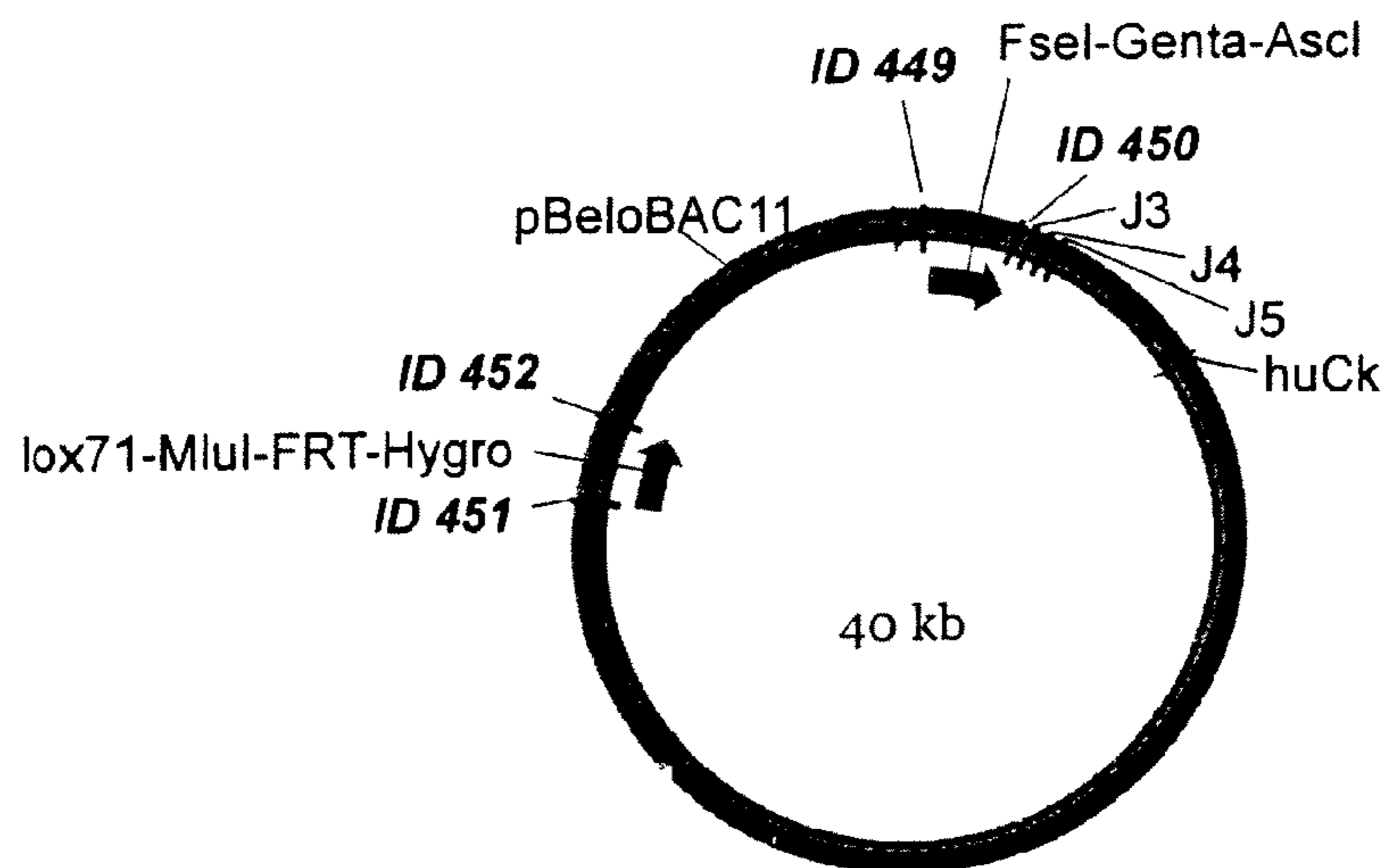


Figure 8

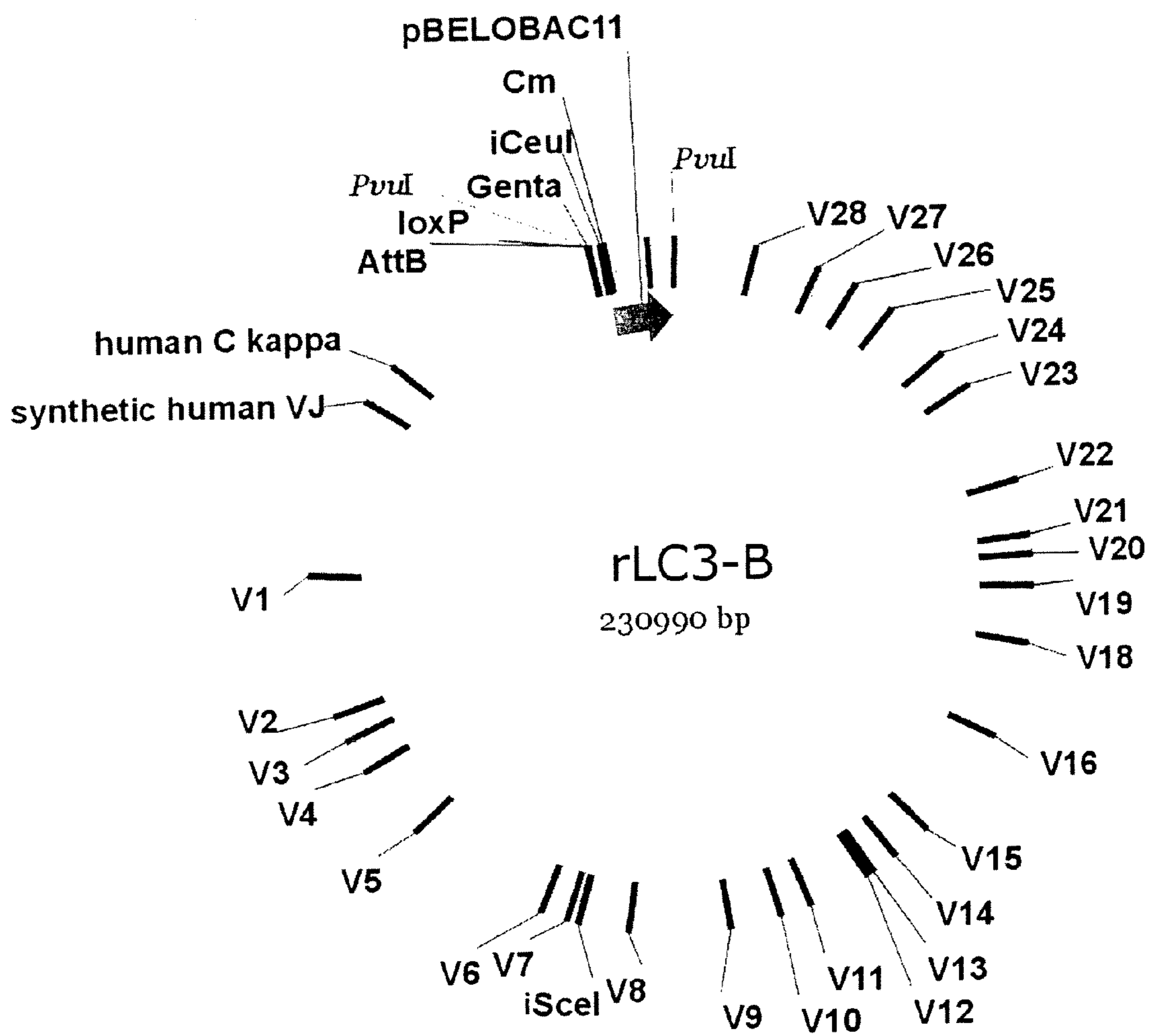


Figure 9a

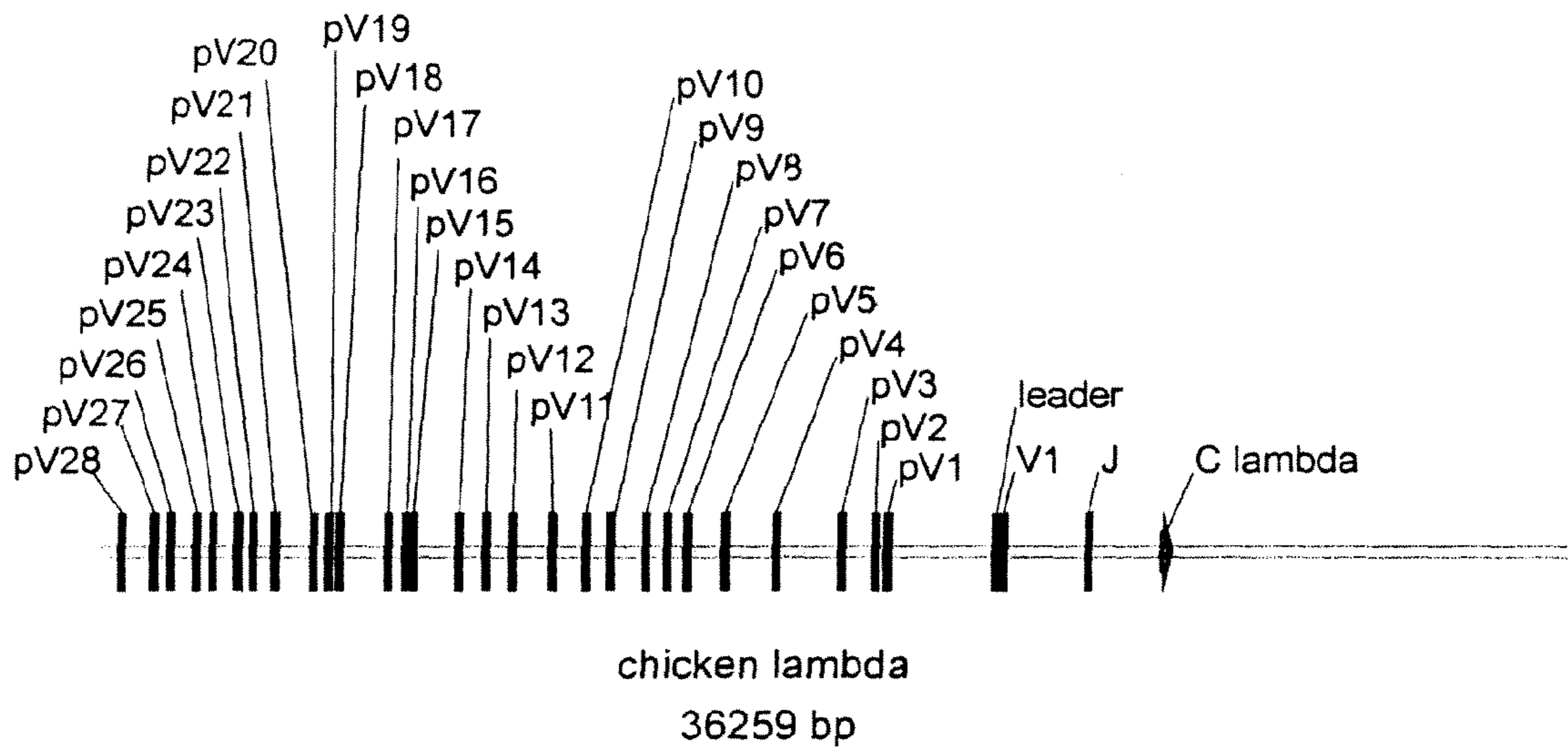


Figure 9b Seq ID 186

agaggatcctattaattgtttgtaattaagaggacaggaattcagtgtcagggggctgctctttgcttccaatgtgtaccactgaa
gcctctctggattatcagagatactcaatggactcttaacagcactgtggcacctgacctctgctgccttcagcctcccttctgg
ctggtgtgcttgtgtgaggagctgtgggaggctggtgcagttctgtgggatgaggttaaggccagctgaatagcctgggggctg
gtcagggggcttagtgagaggagcagggtggggaatattgtgaacagcaggtggtgccactgctattctgaggaaggagc
agcttctgcaaatgcttgcaatgcaggatgacaaagtcttctgctagctctaggcccttgctcatggctgtgatggtgcttaacg
gtgccagagtgcagacaaatagatgtgtacagctgctctgggagtaacaacaacaactatggctggtaccagcagaagacacc
tggcagtgccccctcactgtgatctatcaaacacaaagagaccttcaggtatctttatacttcagctggtaccagcagaagac
ctctggcagtgcatctgtcgtgtgctgaaaccaacagacacaccaacaccacaggacagcttctgagttctccatctctggt
gcagcttcatgagcacgtaaacatcgtggggattcaagctgaggatgtagctgtctatcccttgggcaacagggacagcagtg
gtaagcctggtgaggtgatacaggagatgtcaaaaaaaaaaagaaaaaccaacatgtatggggtaaaaatggaggtctctgc
cctttctctcatggctgagctgggctgtgcagtgaggttaagttcctgtctcgtctgcaaagcactgtctgaatggtctccctattgtg
tgtgtggtgcatgagggattctggggacagaggccattgtctgtccctataaaggcccaagcccctggctgtgtacagtaagt
ccgctggggcacctgagctgtgggcaggtactgccaggagaggggctcaggccatgatccaataccaatggctctgtgtccc
catgctaataagggtatgctgcagccatcactccctcttaaacccccctcttaacttctacagttctaagttctatctctctcca
attaatcatcgtcaccatacagcatcagtgctgtcatcccagttccccagtaatagacagcctcgtcctcgactggaccccagtg
atggtaacgtgcttgtggttctgcctggggagccggagaatcgtgaaggatgttcgagggctctgttgggtgttcatagatcac
agtgcaggggacactgccaggtgcttctgctggtaccagccatagtagctgtatccccggagcaggtgatctcaacggttct
cccgggtcgtgacaccgaggccggctgagtcagaggagagagggggagaaaatggagctcagtgagtgctgcgacacaca
cagccctcagcgtactgcggtgggataacttctgagcacacagtcctcaaatggaacctcaggactctctcatctgcttcaggat
gaacaatgtctatggctgtcccagcagaggactcctggcagtgcccttgttctgtgatatagggggccagcaggagaccctc
agacgttgccttacttccatgttccaaatcctgctgcatgcacaaattaactctcgtggagattcaagccaagggtgagactgtc
tattactgtggtgcttgggaaggcagcagtaatcctggttgggatacaggagatgtgctcccccaaaaaaccccaaaaca
aacaaaaagcatatagggggtaaaaatggagttttctgccatctgtctcacagccaagctgggctgtgcagctgcggcagg
ttcctggcctctctgcaaagctctgttgtgaatggccttccctctctgtaccttgggtgcaggaggagattgtggggacagaag
ccacttctccttgccctacaaaggcacagcactgtgtcctcccagccctctccatgttactgctggcagctcccgtctctctgctt
ctgaagagacatggatgtaggtgaggtctcttctgcaacagtggtgttcatccccacagctgccaggtgagggctgtgtgtgt
cggacatttggctgagctccatttctctctctcctgtccctctccaggttccctggtgcaggcagcgtgactcagccatcctcgggtgc
agcaaacctgggaggaaccgtcagatcacctgctccggggtagcaacaactatggctggatcagcagaagtctcctggca
gtgcccctgtcactgtgatctatcaaacaccaagaggccctcgggtctctctcataacttcttgttctcctcatctggctctgcaaac
acgttaaccatcactgggggtccaagccaaggatgaggctgtctattactgtggtggctgggatagctgctgcctgagtcagtgct
gcctgcaccagggaactgggagaggagaggggggagaaaatggagttcattgagtcctgatatacggagtccttctgtaggc
aatgagataagacagctgagcccacagaccatctttactacagctggtaccagcagaagacgcctggcagtgcatctgtcact
gtgatctatgctgacaccaacagaccaccaaacaccacggggcagcttctcacaatcctacacgttctggtgcagcttcatgagc
acattagccattgtggggactcaagctgaggatgtggctgtctatttctgtggcagcagggacagcagaggttaagcctggtgag
gtgatacaggagatgtcaaaaaaaaaatgaaacaacatgtataggagttaaaatggaggtttctgctctcttctctcacaggtga
gctgggctgtgcagtgagatgagttcctgtcccctctgcaaagcactgtctgaatggtctccctgctgtgtgtgtggtgcatgagg
agagtctgaagacagagggtacttctccctgtccctgagaagctgctgcatggtgtcctcccagccctgctgcatgttacactgt
ctgccctgtccttctgatgccgaagctgcttccatgctgggatgctctgggctgagctctgattctccaaaatggcagtggttcttct
gatggtggtggtattctcattctactgctgtgcaggagggctgtgtgtgtgtggctaccactgagccctgtttctctctctcc
ctctccaggttccctggtgcaggcagcgtgactcagccggcctcgggtgcagcgaacctgggaggaacagtcagatcact
gcaccagggatgacagctggtatggctggcacctgcagtgaggggcattgaggctgggaacaatgcagtgctgtgccttga
ggaacagggataacggggcactggaccagaattgattactgaatgaaaaggttcagtaagagaggacatgcgtgtgatgtac
agtctggcagaagggactggattctccctcagccctgactcaacaagggcagaaagccttggtttgaatcgcagctctgcatgtc
acccccacatctcccacatcactatacagcactattgctcctgtcccaatcaccacagtaatagacagcctcgtcctcgacttg

Figure 9b Seq ID 186 (con't)

gaccccagtgatggtaatgtggccgtggagccggatttgaaccggagaatcgtgaagggatgtccgagggctctgctgctgt
atcataaatcacagtacaggggactgccaggtgatttctgctggatccagccatagtagctgctgctaccagagcaggtgatc
ttgacagtttctcctgggtttgctgacactgaggttggtgagtcagggtacttgggacctgagccagactgtacctgtccctaca
aggtacagcactatgtcatccccagccttcttcatgttctgctgcaagccctgtctctctgctggagctgtctctgtctgggatg
ctctggctctgactctcatcttctaagtggccgtggccatgactgaggttcttctcctttaaagtgggtctcccttcccactgtgt
acgcagagggctgtccatgtgggttattccctgagccccacttctctcctctccctctccaggttccctgggtggaggcagaactc
attcagccgacctgggtgtcagcaaacctggggaaactgtcaagatcacctgcagtggaggatcggccagtggtatggctgg
taccagcagaagtcacctggcagtgctgctgactgtgatcaatgctaaccacacagacccctgaacatcccttcacgattctc
cggctcctctccgtattcacacacacattaacctcactgggggtccaagctgaggatgaggctgtctattactgtggtcctacga
cagcagctatgttggtatagtccagggggagatgaggcgggtgaattgatggccagagctgaaaatgatgggttccaccctct
ccattatgactttgttgatctctctcatgatggcttccctgttttctgtggttggaaaggagcagaaggcactggcaggggtc
ttgctgggagagctggcaatagcagggatagtggatgccccacatggaagaaagggacacagctatggccacttctgatgaga
gggtgccagagcacagcagcacatccctgtgcttcagacatctctgtgccctgggggtgtggaagggaatgcattcatagcatg
gccaggtggagtaagagtaacatgcctactgcagtaattctgcacatagagaaggaagaaagacagaaaatcttctacagtt
ctaagttcctctctcttccaattaatcatcatcaccatacagcttcagtgtctatccagctcccacagtaatagatagcctctg
cgtcggcttgaccccagtgatggtaatgtgcttggagccggatgtggaaccggagaatcgtgaagggtgttcgaggggtc
tctggttgtgctatagatcacagtacaggggactgccaggagacttctgctggtgccagctatagtaataacttccatcatgc
tgccacccccggagcaggtgatctcagcggtcctcccaggttctgacaccgaggccggctgagtcagcgtgcctgcacc
agggaacctggagagggagaggagaggggagaaaaatggagctcagtgaatgtgtgacacacagccttcagcatgctgca
gtgggactgagcaccagcacttctcagctccatttctccaggttccctgggtgcaggcagtgctgactcagctggccttgatgtc
agtgcactcgcgtgttactgattacaggttcagctgctctgggagtagtggcgactatggcagtaactgtcagggcagcaccagc
aaaaggcatctggcagtgctccttctatgtgctgtacctgaacaccaacagacccctcgaacatcccttgcattcaccggttcca
aatctggctccacagccacattaacctcactgggggtccaagccggcgacgaggtgtctattactgtgggagtgaagacagca
gcggtactgggtgccacagtacacagagcaatggagatgtgatacacaacctgtcagtgcagggaacagttggcactggcac
tcactactcctggactcacaactactatggctggtagcagaagtcacctggcagtgcccctgtcactctgatctatgtaaca
ccaacaggccctcgaacatcccttcacgattctccggttctggatccggctccacaaacacattaacctcactgggggtccgagc
cgaggacgaggctgtctattactgtggtggctacgacggcagcactgatgctggtagtgacggggagatgtgaaactgaggta
cagccctgaattaaaacgtctgtttctgccttctcccttatgttgttctgggctgtgactgaggcagtttccctctgatggcc
atggcttgatcatctttctgtcctgctcagtgttggaaggtgactctccagttaccgcgagggaacctgaagatcatttgctc
ctggtgtgacagtagctatggttggctgcatctggcttcaacagcaagttaaccattactcatgttgaaaccaaggatgaggctg
tctaagatgtgtgagctagcatagcagtgaggctggtagagtacaaagggaagcatagctgtgatgtctacttctggagtgcac
tggggcatgaaagcgaaaacagtcacaaagccacaaccacacggagggaacaggaacatgtctacagccataactctgcgtggc
caaatgctgaaagtcagaaaaccagcaggggagcggggctgggtgcagtgcagccaaccttgggggacagctgtagaa
catggaattcaatctcagtggagtcgaaccaattggcaggctgtcagcagaggcattccccagtgtccccttgtttaaagtgaaa
ttacaaaacagctgccaggcaaaaactgtattgatctgcttgagggtaggaaggcctttagacagatgaggataggcaaaa
atcaatgggctgtgtcaaacctgacgttcaagaagctaaagcccagggcctgacttaggtcataaacacaaggcagca
gtgtaggctgaagatggttgattagagggtctgttagaatggttcaattctgtgatcatgaagataactccaacaaaattggt
gtaggatagggataaggtgctctggccccagaatcacctgcaacaaaaatacagcaggaggagcataaacaattgcggccct
gcagaggggcaaggcacttgcctcagccccattagtgaaaggatgcttaagagtctaccggtgtaccatagatcacagtgaca
ggggcaccgccaggagacttctgtgtaccagccataagcactgctactgctgagcaggtgatcttgacggttctctgggt
ttgctgacatgaaggccagctgagtcggtgctgctgcaccagggaaacctggagggggagaggagaaaatggagctcag
taagtgcctgacacgcaacaccagtgccagaagatagcactgatcccaagacaaggagaccatgcatgccaagagggtc
ctcttctctcccacttctctcagtcgggctctagggtctccccctctctctctctctctccttaccctgtacatcagatcccagggc
tttctgcatcttctgctctgcaggcattgcttggggcctgtggcaagaccagggatcagtcactctgggagtacccatgatgat

Figure 9b Seq ID 186 (con't)

ggctggtccaggagaaacctggtagtggctgtgactgtgatccacagtacaccaacagaccctcgaacatccctcacaat
tcctcagttccaccccgcagtgccaccaccagcatatccactatcagtgtctgtcgaagctcccacagtaatagacagcctcg
tcctcggcttggaccccagtgatggtaatgtgccgtggagccggatgtggaaccggagaatcgtgaagggatgcccggagg
tcctcctatcccagtagatcacagtgacaagggcactgccaggagacttctgtggaaccagccatagctatagctgccccca
gagcaggtgatcttgacggtttctcctgggttctgacaccgaggccggctgtgagtcagtgtgcctgcaccagggaaacct
ggagagggagaggagagaggggagaaaacggcaatcagtggtgcctgacatacacagctccccccgcaaagcagcagg
acagagggccccatgccatacaggcccaggtcacctgaactgttggtagccatcactagtaggagagggggcccagaccatgg
tgctactccaacagttgtctatggacccaactcccttttagacccccacccttctacagcacagccacctcctgcaagcctggt
gtccatggatactggccacccccatccatgctccccctgcctcctgtagccatttctctgatctcagaagaccaatcatggttagc
cattactgggatccaaaatgagcacaagactatctactactgcggtagcttgggaaacagcactgatactggtagaatggcacag
ggagatgggaaactgagttacaaacctgagttcaaaatggaagcttctgcacttactgcattatggctggacagggctgcaggg
gctgagtcctgtttctgtcctctctgcacggccctgactgtaaatggcctcctctctctctcttaaccacaatgtacctgtccctgcca
ggcacagcactacgtcaaccctagccctgctccatgttctgtgagtcctatctccttctagagctgcctttgtgctgagatg
ctctgtgctgggctcttggccacaaacatacctatgtctgtgactgaggacccttctcctctgatggtagtgttccccctgcggttgg
ggagggcagggcaaattgggcacctgtttagccccattactccccctctccctatgcaggttccctggtgcaggcagcgcctgact
ctgccagactcattatcagcaaacctgggagaaactgtcaggataacctggtctggaggtagctatgattatggttggcaccagc
agaaatcacctggcagtgcccctgtcactgtgatctataacaacaagagaccctcagacatccctcagattctccggtgc
cctatccggctccacagccacattaaccatcactggggctccgagccgaggacgaggctgtctatttctgtggtgcctgggaaag
cagcagtaacctggtatggtgatagggggc gatgtggggggaaaaaaaaaaaaaaaaaagacacatataggggataaaaat
ggaggtttctgcccctctgtctcacagccaagctgggctgtgcagctgaggcaggttccctggccccctctgcaaagcactcgttgt
gaatggtctccctctgtacagtgcagtgaggaagagataccaggtatgtccagacctgtttgctgttactgctggcagacctatc
ctcctgctttgaagagggcatggatgtctgtgaggccctcatctgccaggggtggtgttctcctatcccaccctgccaggtgagggt
gtgcgtgtcaggcaaacactgagccccatttctccccctctccctctccaggttccctggtgcaggcagcgcctgactcagccggc
ctcagtgacagcaaacctgggaggaaccgtcaagctcacctgctccgggagtagcagtggtatggttatggctggtaccagca
gaagtctcctggcagtgcccctgtcactctgatctatagcaacgacaagagaccctcggacatccctcacaattctccggttcta
aatccggctccacgggcacattaaccatcactggggctcaagccgaggacgaggctgtctatttctgtgggaatgcagacagca
gcagtactgataccacagtgactcagatgtaatacacaacctcccggcagtgcaaggagcagataatacccttaactgtctgtct
tacaggttccacctcaataccccatgctgcagggtcaggtcctcttaggctgccaggtgcaccctgtggtcctccccc
cactaccctacaccaccctttgcagtgcccctgcatgactggggcaaaccttctcactgctccgctcctgctggagggcc
acagccacctggttgatacatcagcatagcaccagttctccccaaagctggccctccctggactctctactgagccccatttctc
ccctctcctctccaggttccctggtgcaggcagcactgactcagccagcctcagtgctcagcaaacctaggagaaactcaggatc
acctgctctgggggtggcagtaacaatgcttatggctggttccagcagaaggcacctggcagtgcccctctcactgtgatctact
ggaatgacaagagacctcaaacatccctcagattctccggttccaagtcggctccacagccacattaaccatcactggggt
ccgagccgaggacgaggctgtctattactgtgggagtgacagcagcagtggtgctggtcccatggtgacacacagcagtgag
gaagcagtgacaaaacctcctgacagtgcaaggagcagccggcaggtttgctgtctctgtttgatggtggcacaggacctc
cacagccctacctcactgtcctgcaatatgggtggctgtgcacgggtcctgaacacgtccaacaagatgcacagcactgcat
cagggccagcccctgcctgtagcagctgatgcccctgtccagcttctgcctgagctgcctctgtgctgggatgctccatgctgag
ctctgatctccaaagtgccatagctgtggctgggatcccttctgctctgatggtggtggcacattgcaggaacagagcccaga
ccagaacagctcagtagcagagccagctccagcagggagagaatggggctgccagcagcaatgtggaacagctggggatgg
cacagagctgtgcctttcagggacaggtacagtgactcagtagccagagtaacctctatacaactacataccaaggaag
gcatttacaagcatggccatgcagagaagataaaatcctcagctctcacagacctgctcagccataaggcatcaagggtgaa
aaacctctatTTTTTTTaaacctgatcaatccatcagttcctcacctcccgtgaggcaataaccagcataagtgtgctgtcgtagcc
accacagaaatagacagcctcgtcctcggcttggaccccagtgatggtaatgtggctgtggagccggatttggaaaccggagaa
tcgtgaagggatgtccgagggtctgtgttgtttatagatcacagtacagggggcactgccaggagacttctgctggaaccag

Figure 9b Seq ID 186 (con't)

ccgtagctgccactacccccggagcaggtgatcttgacggttcctcccaggittgctgacaccgaggccggctgagtcagtgc
gcctgcaccagggaacctggagagggagagaaaacagggtctgtgtgtgtgtgacacgcacagcctcctgtttggcagtgg
ggtgaggctgctgagcacacagacacacatgggacagcaggagaccacatctgctccaggattgacatctatggctgttacc
agcagaagatacctggcaatgccccatcactgggatccatgctaactggttagaccacagcgttctctaccattctctggg
gtccaatccaggcctacacacatgaactgtcacactaactaccactgtggtctaagctgtggagaacactgccattactgcgt
ggcaatgacaggagccaaggcctgaggatggcacttatcaccaggatagcaggacaaggggttcacaagaaaaaagaaag
gttctttttcctctcccgttctctcagtcagctctgtggtctcttctcctctcactgctctccttcacctgtacatcagatcccag
ggcttcgctgcattctgctcctgcaggcattgcctggggccctgtggcaagaccagggatcagtcagtcgggagtagcaatg
agtatggctggtccaggagaaacctggtagtgggtgctgtgactgtgatccacagtgcaccaacggacctcgaacatccctc
acaattctctgttccccatctggctccacgcacgagctaaccatcactgggttccaagctgaggacaaggctgtatattaccgtg
gagactgaagagcagcagtgatgctgtatgttggcaatgttagtaggaacataaacacagccacattcacagtaaagtttcagg
ccaccttcttggtecccagctttgggaattgtgacttatgttactgtttgggaattcaggaggccttgcctatctgaatggccaa
gacctgactgttaccagatcatattccagggaatgagggtgatactgggtatagacccccatccttgcctctccaggttcc
ctggtgcaggcagcgtgactcagccggcctcgggtgcagcaaacccaggagaaaccgtcaagatcacctgctccgggggtg
gcagctatgctggaagtactattatggctggtaccagcagaagtctcctggcagtgcccctgtcactgtgatctatgacaacacc
aacagacctcgaacatccctcagattctccgggtccccatccggctccacaacacattaaccatcactggggtccaagtcg
aggacgaggctatctatttctgtgggagctacgaaggcagcactagtgtgtatggtgacaatgattaaaaggaactgaattaata
aattataagcctgtttctgcccattctctttggccatgcagaactgtggccatagcagtttctgttccatctgcatggccatgcta
tgaatgcactccccccacaccgcagggcacaggagatgtctgaagcacagggatgtgctgctgtgctctggacacctctcatca
gaagtggccatagctgtgctccttcttccatgtggggcatccactatccctgctattgccagcactcccagcaagacctgcca
gtgcttctgctccttcaaccacagcagtggtgctgccccagagctgctcctctggatgctgtgccccacatttctgaacac
aactgccatccagcaatgtgggacagggaaacaccacagcaggaagagacctcaatcacagccatagtgaggcagacag
aaacctgcattttagctctagacatcacatcctccatactcctgtgtctgtacctacaaaactgctgtcataggccccact
aggacaggaacatgaatggggctctgcaaaggcagcgcaccacagcagctgtggaccagagagcagaactgactgcctcaa
cagctccgcccagcaggagatgtgcagcaacaaggaggggaggaatccaacagtgccgccaccagcataaccagcact
gctgtcgtagctcccacagaaatagacagcctcgtcgtcggcttggacccccagtgatggttaatgtgtttgtggagccggatgtg
gaaccggagaatcgtgaagggatgttcgagggctctctgtgcttccatagatcacagtgacaagggcactgccaggagacttct
gctggtaccagccatagtagctgctgctaccacccccggagcaggtgatcttgacggtttctcctgggttctgacaccgaggc
cggctgagtcactgctgcctgcaccagggaaacctggagagggagaggagagaaaatggggctcagtgctgacacacactg
ccctcctcctcctcatggggaggagacccccagagcacaagaacacacagaaatattcaaacaccgacttctaatccatgct
taacagcaactatgactgtacaagcagaagactcctgacaataaccactggcactttgtaatacctccacaataagccccctctc
tctctcctctccaggttccctggtgcaggcagcactgactcagccggcctcgggtgcagcaaacctgggaggaaccgtcaag
atcacctgctccgggggtggcagcagctatggttacagctggcaccagcagaagtctcctggcagtgcccctgtcactgtgatct
atagcaacgacaagagacctcggacatccctcagattctccgggtccgctccacagccacattaaccatcactgg
ggtccaagtcgaggacgaggctgtctattactgtgcgaactcagacagcagcagctgctgttagtgacgggggggagatgtg
aaactctctacagccctgaattaaaacatggatgtttctgccttctccctcaggatcacgctgggctctgtcactcaggcaggtc
ccctctgcatggccacggcttgaacatcttctgttctgctcattgtataggactctccagctacctggaacacccaaaaagatc
atgtgctgcagggttagcagcagctatggttatggctaagcagatgacacctggcagtgccccatcactgtgatctacagtaa
caccagagatcctcatgattctctggttccatcccacgccacacacacacactatcactggggcccatgccaaggatga
ggctgcctattactgtgtaaatgcagacaacagttgtacagctactgcttcccactgacacagagcagcaggtacaactgatgcact
aacctcctgccagtgaggagcagctggctcttttctgtgctctgtttgatggcgggtgctggttctaccatggcccttccctcat
tgccctggactacatgtggcagggcaaaaatcacatcccagcactgacagcagggaaacctgtggaccgctgagcagccagg
ccaaagctccctacttagaggggatgttccatggcacaaggctgggctggctgtgttctcttctggcacgagggcagccaca
gaatctttcagatcaggatgattgtgtgatctgctcctacctcagctcaactggcatcacctacagcacagtgcccaggcctgtgt

Figure 9b Seq ID 186 (con't)

gcaggtggctgtcagagatctccaaggaggagactccatgatctctccgggcagcctggatcagtgtacattacctgcggagt
aaggatgtgactcctgactctggccagacagaacctctgcgttcagctgtgccactgcctcctgtactgggtctgaatgtctgtg
gaaaaagcctggctctgttcaactttgcacctctttcagggttagcgttaaggaggagagagaaaatagggtcagcaattgcc
aacatgcacagccctcaccaggctgtcagatgaggcacgcaattccacaaggacacatggggtagcaggataccacatctgc
tccaggattgtaacaactacggctggtccagcagaagtcacctggcagtgctcctgtcactgtgatctatgtagcaccagtag
acctcaaacatcccctcagatttccgggtccgagtcggctccacagccacattaacctcactggggtccgagccgagga
cgaggctgtclattactgtgggagtgcagacagcagtggtgctggtcccatggtgacacacagcagtgaggaaagtgatgcaca
aacctcctgtccgtgcaaggagcagctggcaggtttgctgtctctgttgatggtggcacaggaccccaccacagccctaccttc
actgacctgcaacatgggtggctgtgcacgggtgctgaacacgtcccaacaagatgcacagcactgcatcaggcccagcccc
tgctgtagcagctgatgccctgtccaacttctgccagagctgcctctgtgctgggatgctccatgctgagctctgatcctccaaa
gtgcccatactgtggtgggagctccttctgctctgatggtggtgacattgcaggaacagagcccagaccagaacatcttagt
aaggaggcagctccagcagcagcagaacggggctgccagcagcagggaatgatgctgtgccacagtaggaacagggacca
agaggatcactcccacgcaataaagtataaaagagagggcattcaaaaacacagccatgcagagcagagagaaacctgact
cagccccacagctcctgctcagccttaagtgcagaaacctccatttcaactcaggtctgtaactcagttccacatcct
ctttgtctctataccagcattagtgtctatcccagccaccacagcctcatcttctgacctagtgacaactaaccacaattggtctt
ccaggggcccagggaatagctgcactgggacaggagctgccagtgaccatgaggtctgcatgagtgggctgtgttgggca
gttcagggtataaagaaaggagttggagtcctatggcatagaccattggcataaggatgtgcatctgctgggatcacaccatgg
cctgtgcccctctcctgctggcagtgctgaccacagctcaggtgccccaggaacaccggtgtgcccagcctttgggactgg
gccccatccccctgctttttaagacttggcatcaattcaccacaacacctctctgcagcaccataccaacatagctgctgtccctg
ctcccacagaaatagacagcctcgtctcggcttggaccccagtgatggttaattgcccattggagccggatttgaaccggag
aatcgtgaaggatgtccgagggctcttctgtctgttaatagatcacagtgacaggggactgccaggtgacttctgctgatacca
gccatagctgccactactcccggagcaggtgatcttgcaggttctcccaggttctgactgagggccggtgagtcactgct
gcctgcaccaggaacctggagaaggagagaaagagaaaatgggctcaacaagagcctgacacacacagccctcccagca
gcagtgggatggggaacagcaccattaaaggaaaagggaactcagccatagccacatcaaggatgagagcccagcccaga
gcagcccagcagagggcagctccatggagcagggctggggatgacaagatgctgtatctttacaaggataggtacagtggtg
gtaagtgtcagagtaatctctcatalacttaggtacagacagggagggcatttcaaacacggccacgcagagaggacagaaac
ctgctcagccccacagcgtgataagccattactcagtaagtgcagaaaagtttcaattttaactcaggcctgttactcagtttcc
atctccctgtgactgatccccaaacttggactccagtgaaaggtaacctatgaccccttctgtgagctgagaaaaggagtgtag
tgagcaggagagcactgcaataggagctgatagtgatcacacaggcctgtactatgggcagatgggagtttaagagtgaattg
cggctccatggcatatgcctgtcagcatagggatgctggaggaattgcaccttggcatgagccccctcctcctcctggtgatgcttgc
cacagctctgactgctgggaaggctcactgcaccttgtgtcagtgaaactgcaataagctatcaagatttgalataatttgc
ctctgatctacttgtgagaccacacctggagtagctcctcagttctggtaccaccaacacaaaaaggacatggagctgctggag
cagggccagagggagggccatgaagatgaacagagggctgaagcacctcccctatggggacaggctgagagacatggggct
tttgagactggagaagagaaggctccatgggaccttccagtgctcctccaatacttgaagggtccttgggaaagctggggagg
gacttctataaggacatataagcaacaggacaaaaggaaagggttttaactggaagaaagtagatttagctagatagtaacaa
gaaattctttacagtaaggatcacgagacactgaaacaggttgcacaaagcagctgtggatgccccctccttggaggttccag
gccaggtggatggggctttgagcaacctgttctagttagaggtgacctgcctacagcaggggagttgaacctagatggtctt
aaaggtcccttcaacccaaatcattctatggcaattctatagagaacacctatgctgagagtagctacaagatggctggttctg
gcagtgccccgtctctgtgatctatagcagcaccacagacctcaaacctcctcagattctccgggtccaaatctggctcca
cacacacattaacctcactggggtccaagccgatgacgaggtgtctattactgtgggagcacagacagcagctatgttggat
cacagtgcacatggagcaattaaaaatgatgcataacctttgcacatctgtaatgctataaggtagagagaaagtaagcaataatag
cagagcagcacaatcctaggctacagcaaatgagaacaagcccagatgcagccacagaaataagaaaaaaaactgcttac
ctttgaaagcctcatatagacagggatctccagcaagatacccacacctccactgccaaccttaaatgagggctgggatggg
ctggatcctggctccaccttcaatcactcaggtacattgcacacacctgagctccccctggctggccctgccttcccaccaggt

Figure 9b Seq ID 186 (con't)

gctcaatcactgcttgggctgtgactcagcatctccactacagcaccacaggagcgggtgctcgtctgctgttcctatgtggg
tgcgggggacacctgaaggcatgagtgagcagtgaggctcccagctctatccatggtgaacagacattatgtttatgctttaca
gcagcatgaatctggcagtggtgttattgctgtgatgctgctgtctatgctcccacagtaatagatagcctcgtcctcggcttgac
cccagtgatggttaatgtgttgggagccggatccagaaccggagaatcgtgaaggatgtccgagggtctgttattgccattat
agatcacagtacaggggactgccaggcatagtcgctgtaacccccggagcaggtgatctcagcggttcctcccaggtttgc
gacaccgaggccggctgagtcagcgcctgcaccagggaaacctggagagggagagaggagaggggagaaaatggatc
tgagcaattgctgacacacacagcccccccagactgtgggatgaggccctgtgcccgcagtcacatgtggaatatcaagaca
cacacatctatgacaatcacaatctgattatcaaccactatggctggtaccagcagaaggcacctagcagtgccctgtcactctg
atctactatgatgatgagagaccctcgaacatcccttcacgattctccggttccaaatccggctccacacacacattaacctact
ggggtccaagccgacgacgaggctgtctattactgtgggaatgaagacagcagcggactggtgccccagtacacagagca
atggagatatgatacagacacctgacatctcaggagcagctggtcattatcctggtgacacaagtcccaggcatggcccagca
ctgtgctgcaggtggctatgccgggtgccctgctctgccctcctgtagagcctcaggactgtgccatcactcaggaggagat
ggtcccagcagcctgtccctgccctgcactgacttagctcctggacccatctcctgctgcccaccatattgctccctgtgtt
gctgttgagggtgcttctgctcactggtttctcccttctggagggtggccaaaagccgggcccctgtgcaatcctggtgcataa
ataccttatggcccctaagtagggcaggtgtgggacacgctctggcacctggggtgtgtgcaagtgtcaggaagacctgcag
gcacaggtggcagtgagggtctctggctgtgctcagcagcagctgcctgggtaagggtagtactctgtgcatgaacaatg
ctgcagggtcagctctgctcagaccacgacctggcaccacagagacctgcctggctctgtggtcatgtaaacctttacagg
agctcaagacaaggctgtttattactgctctggcaggaaagaagcactggccatggtcatagagagttccagcaacaggaaagt
gagagcccaagctgctgaggtaccagggtcctcagggtgcctgctgcagcagcttgacacagtcgaggaacagcaattgtac
ctgtgtggtggatcaggctgtgctgcctgtgaacctattctagcacatctgtcacctctgtccactcacagggataccaccctga
gaccctaccccatcagcctctgtgtgggataggtgtgggcccagggtctgttgacaggagatagaggcctggggag
gagggaaagcattgaggtggtgtgataccagggatgtgagcccaagcaagagatcagcagagcaaggaggaagaattgca
gggtgtgggctggggaagccccagatggctggagctggtggggccactggagatctcctcctccatcctgctccatgctg
gggcagctgctgaggtgaccagggtcctcccgggcacgttgtgaaggtcaccaggatggagacttcagagctatctggg
acacgtgttttaacgtttgatcagccttgacgcaaaacagtgtttctctattgaacagtggtcaaaaaaacgatcagtgccctgg
aacacatctctacgaagaaagggtgcgagagctgggtctggagaagagaaggctctggggcaacctccctgtggcctgtcag
tgcttaaaggaggctcaagagaaagatggagagagactttatacacggagctgtagcaatatgacaaggagaaatggttaaaa
tggaagagggttagattaggttagctatcaagaagaattcttactcagaggtgatgaagcactgtagcaggtgcccagaga
agtattggatgcttccctggcagggcccaaggccagactggatgggtctgggcagcctgtctggtgggagatgtccctgc
ctgtggcatggctggaaatggtagccttgaggtccctctcagcccaatcattctatgttctgtggtccctgcattcagcctt
gcctgtgactctggtgccactgttgacaatttactcagctgcgtttcatcctgccccaaagcagtaatcccatggataagcaa
tgctctgaggccttcaccacctcagggtcctgctgacctagcggcctccctggcagcatgagcccttgttgcactgct
gctgctgagggtcctgccagccccagggtcttctgccaggcactgccacctgctaaaaccagcctgccatggcagg
gcacgcagctgtgagggatggaacggggcaagggcagtgctgcaggcagtggtcaagaaacgtttagatgttactgagg
gatgtggttagtggaataactggtgataggtggatggtggactggaggatcttagaggctttccaaccttggtgattatgtga
ttctatgaaggagccatagcctgctccatcatgccccgccagtcggctctcccagcactttggttccgcagcatccaggcc
cgctctggggccgggtgtggcctgggtgctgccacgtgtccccgctgcaaggcctggccaaccccgctgtgccacaatgcag
gggctgggctaggagtgggcaggaggtgtggggagaggcgtaggacgtggctgggagcgcaggaggtatttgcattagg
ggggcgtgctgcggaaggacgcgggtataaaaggcctcaggtccccggcacagccccatggcgtggggacacacagc
tgctgggattccgccatggcctgggtcctctcctcctggcgggtgctgccccacacctcaggtactcgttgcgcccggctgggg
actgtgggcacgggctctgtccattgctgcgcgggcagggtgtgctgctgcccggcgtcactgattgccgtttctccctct
ctcctcctcctcaggttccctggtgcaggcagcgtgactcagcgtcctcgggtgcagcgaaccgggagaaaccgtcaa
gatcactgctccgggataggagctactatggctggtaccagcagaaggcacctggcagtgcccctgtcactctgatctatga
caacaccaacagacctcgaacatcccttcacgattctccggttccaaatccggctccacagccacattaacctactggggtc

Figure 9b Seq ID 186 (con't)

caagccgacgacgaggctgtctattactgtgggagtgacagacagcagcagctactgctgcacggtgacacaaagcaatgggga
aatgatacaaaaacctcctgccagtgcaaggagcagctgatggttttactgtctctgtcttacaagtcccacctccattcctgccct
gtgctgcagggcccaggtcctctgcattcccaggctgcaccccaggctccagctggctgectcctgccatccaacatccacca
ttttagtgcctcctgcacaccaggctggcagctcctcatctgctcctgctcccactgagaccacctgccagcccatgctgga
gggccaccaccactcaattgcaactgcacatcagcacagcaccagttctctacatgtgctcctctgaggaaaagagctggacttct
aagcacccttagtgtactcacctaaaatgaaactgaaacccataaaaagtctcagaaatacccagaaactgtcatccatagtatat
ggcatcaacttagctatatactttcatagatagtttcaatgagttctatcacttgcaccggctcactgcacctaagcctcccagag
gaaaaccaaacctttgaaacagaaagtacttccaaccacaaaatctttgatgcatcattcattttctagtactcaggagattacca
actctgctctttctataaccacttgcacaaaccacagcatttgcataattgtgagtttttaagccagtgccttggctctggcagct
ctggttgggcatcaacatcctcttcacatctttctctctcttggaggagatgtgggcagcatgtgcagcgcagtggtgcaccaat
gggataaagctgggcccagtgacctcgaaggacaacatcagcgagagtaaggccatcaaacagacaggcctgaattggag
gggctgcactaaaactggatgacaggcaagaacattatttgggtgggtcttggcaggtgacatgacattggccaagacctga
ccaacaatggcacagctactggatcccttggagaggtgaggaggacacacagacaccatctacactctgtgaccacagc
aggcagcagggtcagccacacatgagcgggcatgggcccgtgcttgggctgtttctgcacggccatgtatcactgcaggtatt
tccccgggtgggacctcaggaatcaactgatcgtcctcaggaagtcgctctgtgtcacttacagtcctccccagcagtaagtgggt
gctcaggctctcagccttttttttttttgggtgggtttgttcttgcacactttggctgtggcagtacatccttggctgcctaac
cacactgaaatgtactcatgcacctttccctgatttgcagaaagaagggtgtatggtctgaatgctctgtgctgttgaagtctctct
ggttctcgaatctgcagagtcctccccgactgtgctcttggactgaacatcctgttcaccgtgtcagaaggcagctgggggg
ctggaggcagtagtgcgtggcaccaggatcccctggggcatttctgctgcggcgcctgggtgctccacgtggctgccaggg
ggacacacctcctgtagcagccccagcaccaccatcctgctgtgtccatccgaccttgatcctactgtgtccagcccacgt
ctcccttctgcttgcctgtcagaagaacacttccatccatcacttctgaccacccccctcactcctgccgcctccacaggaccag
aactgcccctgccacgaagtgggaggttttgcattgctccgtatcactgtgtggtatatttggggccgggacaacctgacctc
ctaggtgagtcgctgacctgctcggcttttctccccatcgtgaaattgtgacattttgtcatttttgggtgatttgggggttttctt
ggacttggcggcaggctggggtctgccaccggcgcagggccgggactcagcgcggcagcctgggctgagtcttgcctcca
ccgagccggagggtccgggtgtgcgccatggaggacttaggggtattttgtcaatggaaaagtcttaaaattgaccagaaaatgt
gcccgaggtctgtctctgccacacgatttcagaaattgtgtctaggtcagatgagaagacagttttgtctttgtcagaaattagttg
gagttgttagctctcctcttagctcctaaggactaagaccttgcctccggctctcactggggactcttggctccagtgcc
atggggagcccaagtgtcactgacacgggtgctccttgggggtgaaattcagttttcagctgtccaggttaagtgtgtcaaaaaatg
gaggttattctgtgaaaaagctgagaggaatatttgcatttttggaaatatatatataataataataaattattat
atattttatatatatataataaataatgatttctctcttcttttatatatataataataataataatatttctcctcttctttatata
tttataggagagaatctatattttggccaatttggccaatitctctctctctcttttatatatatatatatatataatgtaatttatata
atatataaattatatttatttatttatttattatagatttttatatacatatatataataaatttggccaatttgaagaaattggcca
aaaaaaccatctgaaccaaattacttagggcataagtccaaaaaaaagtgcagttcaagaattcctcgtcggaaagttttaat
gggggcagttctcccatttctgggtgtagatatgagtgaaatagatatgtatgtagatatagatttggctatagtttgttgatga
acagctgaggaagagtaaatcttgcctcctcatggcaatttttaattctacttctctgagacaagtctgaacatgccttgaggtag
aaagagcccgaaaatgagtgcttttcttgaatttttaattaaataaaataagccttacagcaggaaagaaaggggtgatg
gcagctaatcggagttccaaagctgaaccacgtcaccacacacgtgggtgaaatttgggtgtgtatataataataataatg
ataaaaagaaaatgcctatatataaaaaaattgtatgtatattatagatttattatagatatgtacataagatataacacgtataca
tatatatgtagctgtgtatgctttgtataaattttatagatatgatgtatattatgcatgggttatatactataatattttatctgta
gaaggaaactacagtataaaatgcatafatgcgtatatacacatatttatatatatgtgtatataatgtatacgcacacgtataacat
ccatgaaaatagatatggatgggtggatgtgtttgtttacagagggtgcatgtgtgtctgtacatacacagccatacacgcgtgtg
gccgctctgccctctcttgcaggccagcccaagggtggccccaccatcaccctctcccaccgtcaaggaggagctgaacg
aagccaccaaggccacctgggtgtcctgataaacgacttctacccagcccagtgactgtggattgggtgatcgtggctcca
cccgctctggcgagaccacagcaccacagcggcagagcaacagccagttatggccagcagctacctgtcactgtctgccag

Figure 9b Seq ID 186 (con't)

cgactggtaagccacgagacctacacctgcagggtcacacacaacggcacctctatcacgaagacctgaagaggtccgag
tgctaatagtcccactggggatgcaatgtgaggacagtggttccacacctccctgtccctctgggccgctgctggtggcagcag
ccctcactcccactcagatgtccccaccgtgccccatcaccacctctgctgtcactcctcttgcctcactctccaggtg
tcacattaataaacacgacctgaactagtgtgactctgcatccatgtctctgtgtcctttgcgtgctgtctgcatctcacacagg
gggtcggccccagtatggggaagggtggggggcgcatacacacatattgtaatggtggggacgggggggggggggtgg
gggggtcaacagatcagcactggagacctgggtgtataacctggcaccaccaacatctaaggcagggtgcttggggcaattt
ggggcagtttaaggtctgtgctggcactgagcacgtggctgtggccgtgctgtcctcactcccaccactacggctgtgcgcc
aggtccctagcagagattgctttatgctgggaacagggggagtctggctctgtcccttgattcagacacctggtgccccct
gggtgggatgtcagtgtgaatactccttgtgccctgtgcctgcagcagcctgacctccacacaccacacgccttgtgtgcacc
ccacctgtcactatecctctccccgctccccaggagatttgcagtggccccgtagggcagcttttagcacagccccagc
agcaagcaagcagaaagcactgctgtgcacagctgtcagctgtgtgtttgctgaggaggatctgtctttgctgaggccatca
gtcttgcctgtcaacctccatcgtgctgccacctcaacacatctacctatctacaccaacatctccattcatccca
cccacccaacatgtccatccatcacacacctccatccaacccgcacactccagcactccaatcattccatctacaccacct
gctgatctgctacagccactccaacgcaaccgtccattccatctacaccaatgtccatccatcccagccactccagcactccag
ccatcccaccaccctatgtctccatccagccactggtgggggtgcaggacatggggccagctctactgtcaggactggggtttt
gcatggccccataccacttctgcagaagagacgactgaaagtttggtgaccatttctccacggtagagttgtggcagttctgt
aatttagggcttttatccagtttgagatgggctgggatctcccagctccatggcaggcattcatgacactgggttagtatctgat
gggtgggatgtggctgaacttcatttcttccccagtgacaaagttttgcagttgaatatgaattcctgcttctgctctatgagttgt
ttttcccaggacgtacacagggaatcagcagcttctcctctgccatgtgtgactctctgccacacaggactgtgctgtcc
tcatgccccgcgccccaaattgctgccctctgccatgctgccaagctgagccctgcaggctgccatgctggattgacatgagc
cctgagattggtacagaaatggtgatttggggtttcttgcactcaggaagctgaaggctcaatgctcagtgatggattacaaa
ctgtgccctgaggcagctgctcatgctggataaagtcactggagcacaggggaaccaggcgtgggcagggatttctcatgggc
cccactggaaagctgcaggctgcaaacctggctctgccttcacgcctcaccctcatgaggacaacctcactaattattgattaa
agatttgcataaacctctccagaagcaacaacctgaggagcatgtgctgaattatacatcacagctccacggccctgcct
catagcagggtgcatggcaccacagtgccactcagaggaccacagggtgagacagccgggtctggtggtggggaca
cagctgagcataggatgagccccgggcagtgctgggcttctaatgagcagaagtatggatagaaagcaacccagggc
tccgtaccagctgcagctcttctctgtcgtccttggtaaaccttaaacagctgccttttttctcttcttcttcttctggcttgcatt
aattcaaacgagagagacctaattagtaaatgagatgcttcaggaaggcttaattagctgcagatggaggcaggcagtgcta
tcgtggggcctggatgcacagggggctgcatatctcactagcagaatacactcaggctgggtcccaccacattcatcccc
agaccagagggaatatgctctgtccccacacatcgtcccacttgcagccgttagccccaacatcccaccagcacacggg
gctcagcacgcctggcgacatggcatcagcagagcaggccgatggtacagctccatcagcacagctggggccacacaaag
agctgggttactgtgggcagcaggctgaaacccgaaaacaagagctgggggctcagaatagccccgggagcaggcagggc
ctgggggtgagggaagcaccaggcccagggccacacagccctccaggaaggcacagcgtgtcagggtgcagcacgct
cagccccacctgcagctgtcggccggggcatccccaaagctaaatttactctcagctccaatcagaaactgaagctgaggg
gcccacaccggccaaaaaaggaaacgaaacagctctccagaagcactgacgtgtgaagcagagcgagcggccgcgcaaac
cggccgcatgtcacacacctcagggtggggcttggcagactgagcttctgctgctcgggggtgggtgcccacggcctgggc
acatgggatgggtacacacgtacacacacttgcacaccacacccccaacacttcaggatgctggtgcagatgggtgcccc
ccaggctgacccccacgcatgggcctggccccacactgctccatcctgtctctgtccccatgtgccacctgcccgtcc
caccacgctcaccacaatcctgagttaatcccacgactcctgctgcttccagcgtccatggcagactggagatgccccaaat
gcagagcaggttccctgaatctgagagatgaaatggagtattgggtgtccctgcggcggagccccagctgtaggaagctca
gagccatcacacagcaattaaagggaattaaatcaataaatgttttagcgggctcagctgccagcaccacctgaccga
aacagcccgttgcaagaggagagcatttgcattggctgtggcaaacagcaaccgcctgtgtgcagctgggatggtgttatc
tggaatgtacgcagcccaggagggtaaacagctccaaactgagaccccgaactgtccacaggttgaacaggctgacat
aaacaccttgcctgggaaataattatcacctcaatatagcaggttaataaaaactcccacggagctacacacctgc

Figure 9b Seq ID 186 (con't)

tttgaagggaagcagacactgtttctgcttgatggtggctgtaggaagccatgtttcccgatgcaggaggccacaaagcact
gacaacacaatgtgagctgagcttcgcccctgtttaagccccacctcagggtgtggtctcggagcaggcaggacgcagg
gtggcaccgggctgggtgacatgggctggctcctgggggtgtctcactgagctctttggggagggttgagccctggggcaatc
acagcacacacagaggaggtgggggatgcagccagcagctgccctgcactaagaaaaccccatccgtgggctttcagatg
gccttccatctctgcagcctctgcatgggctgaacacaaggtttaagtgtttctgccatgttttggggcatgtttggaggggca
gcgtgggcccgggcatacgggtaactgccacgtgccgccagccccacagctgagcctgcactctcccagatgtgctgaccgca
gccacgggggcaacagtttctctgtaaaaattgtagccgggaagaaaacacgtggcaacttcggccaaacagcagctggag
gacaggaatagccgtggccacggcacgctctgcttctcggcacaaacattccagtagctggcaccacgagcggcgtgcc
ggcacagcagcaagcagagccaggagcaggaaatgctgatttgggccccatttggccatggctgagagaagaggctccag
ggagctggcagcttggccccaaagctgtggcctggggaaatgatggggaggggattgccactgccaccctgcagagcagg
ctctggctccatctcactgcagggcaccagggcgtttgactgcagcaattcacagaaacattgaaatggctcctgcctgttcaa
catcttcatcagtgacctggatgaggggacagcatccaccatcagcgggttactgatcatatgaagtcgggaggagtggctga
cgcaccacaaggctgtgctgccattcaacaggacgtggacagactggagagctggacagggaggaaccaatgaggtcaa
caatggcaagttaggatctacacctgggaaggaataacagcatgcatcagttcagggttaggggctgagctgctgcagatgagc
tctgagagaaggacctgagcatcctgctggacagcaggctggctgtgagccaccggtgtgccctggtggccaagaaggccag
tggatcctggggagcaccgcaatgagagtgggcagcaggcgaggagggtgaggctgcatttgagcaccgtgccagttc
tgggctcctcagttcaaggcagacagggaactgctggagagagcccagcagaggggctgcaatgatgaaggtcctggag
catcgcctgtatgagaaaaggctgagggacctgggattgtcagcttggagaagagaagactacagggcaggagccaagtgg
atagggccgggctcttccagcagtgcccagtgacaggacaaggggcagcaggcacaaagtggaacataagaagtccatct
gaacatgaggaaaaactgcctcgtttgaggggtgtctgagcactggaagaagctgccagagaggtggtggagtctcctctgg
agataatcagagcctggcaggacacttttctgtagtaacctactgtaggaacctgacgcagcagaggggtcggactggagg
atctccggaggtctcttcaaccctacagttccatgaaataacctcaaacactgccaagcagctgctaaggcaaggtaacatt
gtaaactgaaacagggtgggtttaagttagatgtaaagaagaactcttactcagagggtggcgaggccctggcacaggctgc
ccatggaggctgcgggtgccccatccctggcagtgcccaaggcaagagcccagcagtgaccacagccccacaaggacgag
cgtggcccctcgtatctcagctaccctgccccagctcaactcccacctccggcacagcggggcacacagccgggcccctgtg
cttatggagccccttggggcaggtcagcactcacacctccaaacacagccgtggctcccaccggaggcagctggatctcggc
agccataaccaagcagggccatgcgggggtgacaccgggggtccccaccctgtggggcagcgtatgggctgggcccctg
ctccagctcgcagcgtgcatgggaaccatcaccagacaccatctagaccacctgcagccctaagctgcctcacagcaggga
ttgctcctcaccgtgaccccgtgcccatttccatcacttacggggctgggagtgctggacctgggcacattaacgaggat
tccccgctctgccctcgtttgctccgagccgtggggctgtgtagtgacagacacagctgcagcctaaaattagcacctgggaaag
gccccatgctgcaccgcagggctgagatgtgccacgtccccatggccggagctggggaaggcaacgtggccctgtgcg
tgtgcacgctgagcacaaggacacgtgctgggccaggattgtctccccggggctcacgctatgtgcaccccgtgctgtcca
tcccccccgcagccccagctccccacggccgcagccgctgcatccctgcaacggcaccgcacagagacacggagcc
agggggccgcacacggggccaggagctcactttattgcagccctgacagccccacggcccagcccatagcggggctgccac
atcctcaccggccgacggccccagctgctccttaccatttccccatcaccataaaccagaagccgcctcaccgctacgc
ggagcgggcagcagggaacccgggcccctaagggggagacgagagggggccgagcaggggcaggaggagcagcaggg
cgagggggcagcggggcaccacagctggccgtggcatcccgggaggagaagaccttgcggctgcggagcgggtgtgg
cggacggaaattgttgctatctcaggggctcagcgcggcggggaagtgacgggtgctgacaaacgcctgcagctgc
ggggagagcaccgcggggcggcagccgtgaggcgtagggcgaagcggggcacacgcgtggctgctgccgggcaagc
gcagcgcaggagccccgtcttccccctaccggcagcacacggctctgcacacaccgcgttcgtccgcctcgcagccgac
gctgcaggaagcccagccgagcgttacagagcggccgggaaatgcatctgctgaggtgcccgggcaatgcagaactcatc
catccccatccattaccagctcccctccaaacccccatgccatccggcgaccaccaccctctcttgggtgcccctctca
agctctccatccccacattcctacagatgtccccttactttgctgcaaggtgcaagaaaacgcacagggaccgggggtgctca
cagcacggcttggccagacgggcccctccatccatggcagcagggccgaggaatccattacctgctcctgctgatgcc

Figure 9b Seq ID 186 (con't)

acaggctcctcaaacacggtccagatgacggcctcgtgcagtcaggggtgtcagggagccctggtagcggtagtaccggg
acagctgtgcaacgtgcggcagcagcgtgccaaaggcggaaagggtggaggccaggtccactgcctgccctgcacagcaggac
atgggcactcagccccgctgccctggcacgccccagggtctctccccctcccagccgtgccccattgcagagcaccaggga
cctcaccagcatgggagatgttcctcagtcccccaatgatggtgttagttggagtttggggcttctgagacctgcaaagagcga
gtgggccctgcagctgtgctcatttaaggccaacgcagtcagcaccatccatgcactttgcagccaacctggaagaagca
gcccagcacagccagcccgttgggtgcccttggcctccccagggtgcggacttgacgttgatgtggacgatgtgcagct
ggagaaagaaagcacggagggtcagcagaggcaccggcctgccagcagccaggcaccaggatgggaaatcaaagg
gccgtggtgctaaggggcttctgcctacctccatggggagctgctgccatccacgggtgtgctcagagccattcgtagacagg
ctgccccagtggaagtgcagctgcagtgacggtagcggccccggaggccccgccactgatggcaatgtgctcagcacc
ggctcgtctccaggctcagcatcactgcaaacgataggagtgggacaggactgggggcccgtggggcagcactgctgcaga
gctgagccctggctggagcagaacaccaccgctgtgccatcgttcagcagccgccacttgccggggggtgctggtcata
gccctcaaagatgatgtcccaaggctgccgtcccgtgcagccagcggcgtgatgtaaccggagactgctgtcaccgc
cgcaggtggcctcagctcctcagtggtgggctggggcctgcggggagcagggccatgggggcaccgcagagccaccagcac
cacccccaggggggacacagagccaagggcagccctgtccccaccaggacctgccccatctccagcccttctccccgt
tactccaggaaggtttacttgaaacctgtggagctgtaaccgccctgtgactccccattaccaccgcttcattaaggat
gggtgccctcagcagctgcctgttctgtgctgccccaaaccgccccccagccccagcacggtttaattgctgctggagccggc
tctgtgccgggcagtgaggctctgagcgtgctggacgcagctgacctgcagcacagtgagcacgcacctgagggctataaa
gcaattgggctgtaactgctgattacatgctccctgaccttaattaacctttacaagctattatacgtgcgtttcaaagtcccc
gggactggggaggagggcaccgaacagagctgtgaccgtccccactcaccgacttcgggtcctgcgagtcgtagcacca
ctgccctgcgggagagagctgtagtcaggggctgcagaggccccaaagccccccatactgcggatctctgcacagcacac
agcgtgaccgtgggcagaggtgcgccacaccgcccggagcagcgtacgcggagccgcttccctccacctcgtgctgt
cagtgacgggacacacagctctgctgtgtgggagcgggtgaggagcgcggctgctgcccacggccgctctgctccgctca
tgtccaccaacctatccaatccccgtgtgggaactcagccaccaccagcactccctgcctacagggatggttccccgctgacc
ccgtgctctctccagccacaggccgcagggcagcgtgcgtgcaccgctgtgctgctggcttgcacatcctcaccacgc
tgctatgtaggctgcagccacagggtgggaacagggactgtcccagggcagtgacttcaggcttctccatccagcgg
gaactgagcctcctgcacacctcagagccacaaagcgtgccctcaccactgtgagcatccctggcgtgagccagggtgg
gcgctctgctctatagaagggtgctgcgactccagccccattgctctgaggtgtcctcagtggcatttccctgcttatgagt
acatggggcagaggggtgaaacctcaatcccagctgctgattgaccgtccgacatcccagacacctcccagcagggtga
cacctccctgagccggcagcacggcggggacaggggtgtcccacggggagggcacagaacacagctcctgggctgcacc
acatctccgagcggcacagacatgcattcccccaacatgtgggaaagggaatagagtgctggcactgccgtacccccctcc
ggcaccatgctacccataacccccaaagccacctgcactccgtgccatccctccgcagccccaccacgacggtgcagcag
acgggcagcgcac

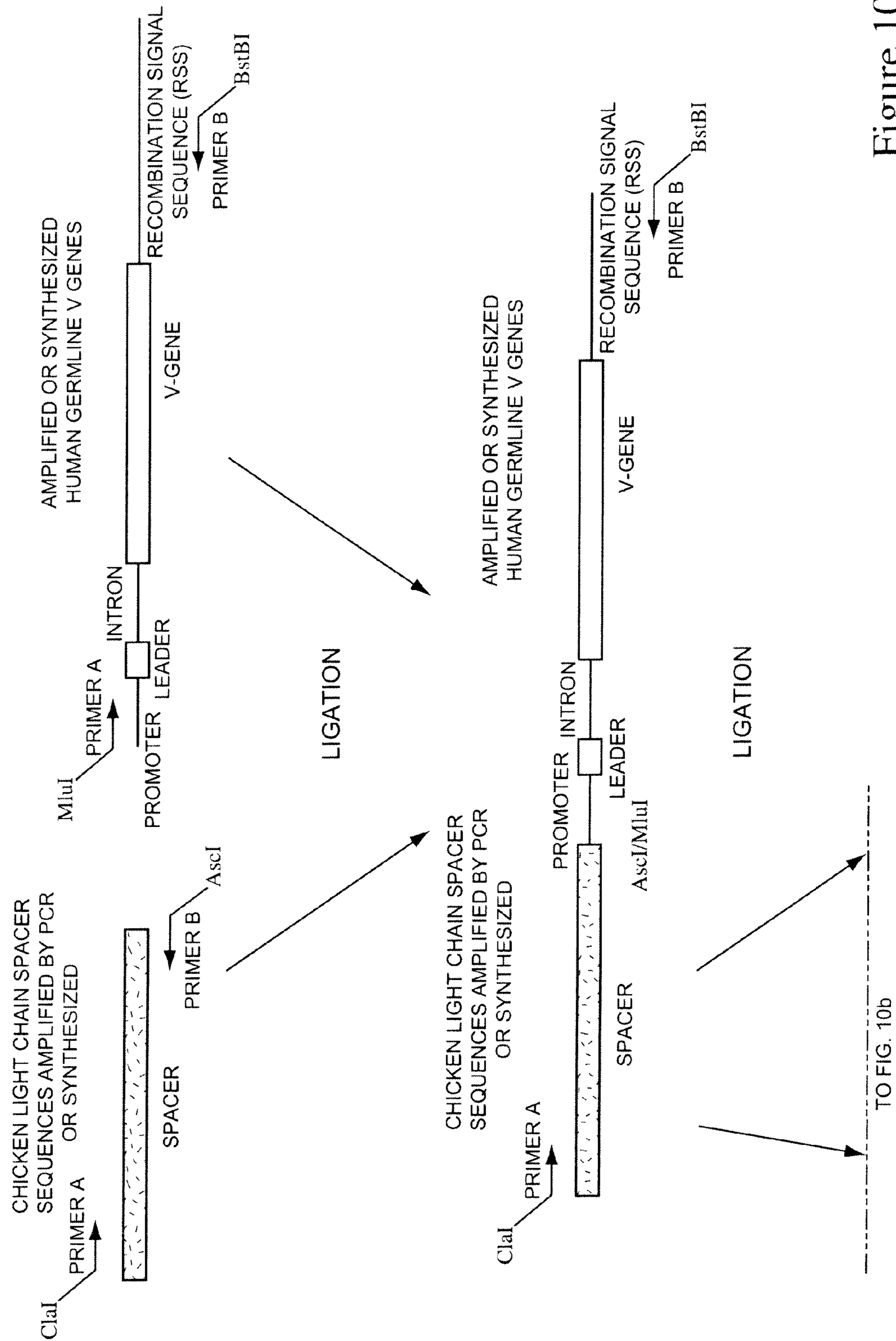


Figure 10a

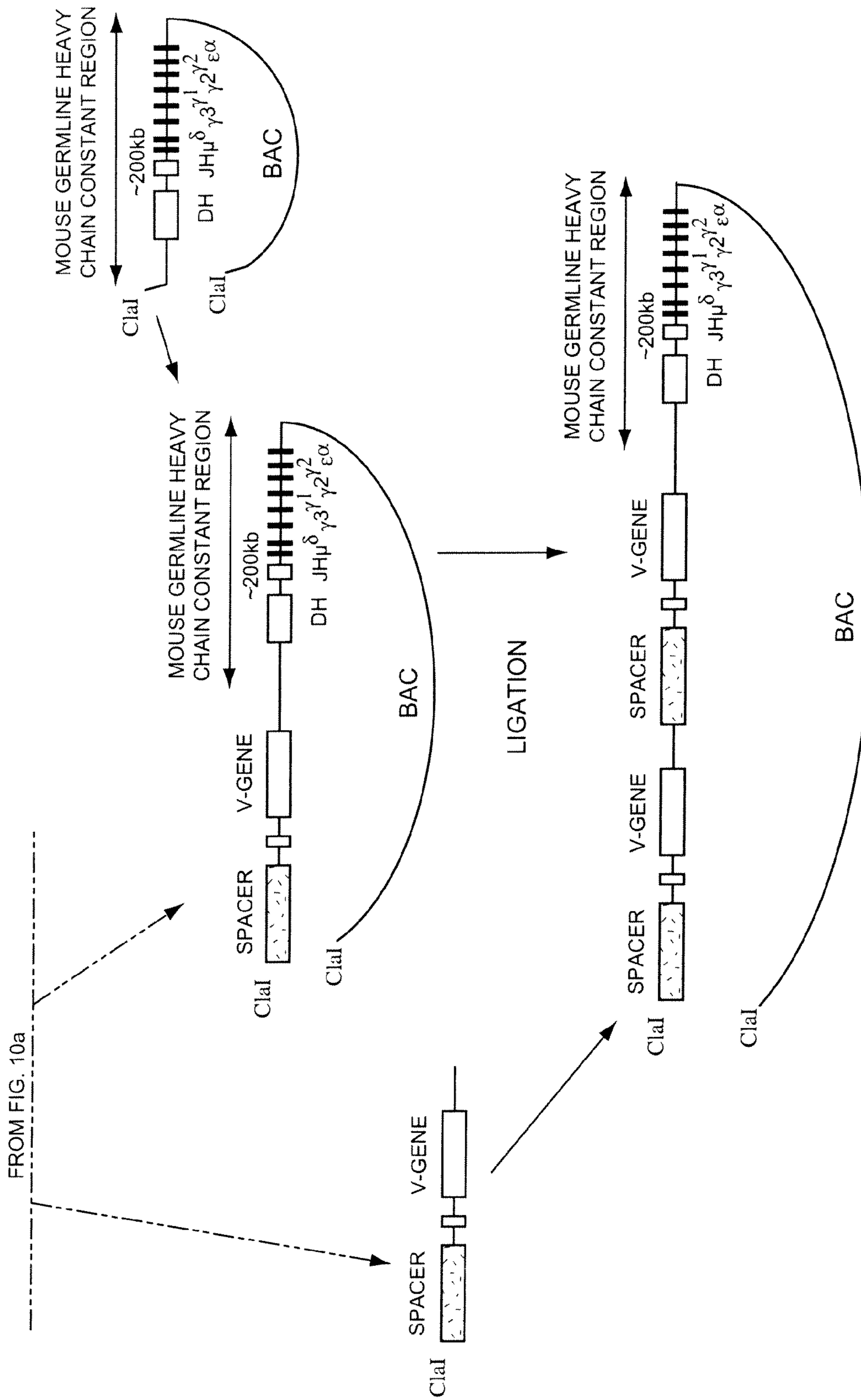


Figure 10b

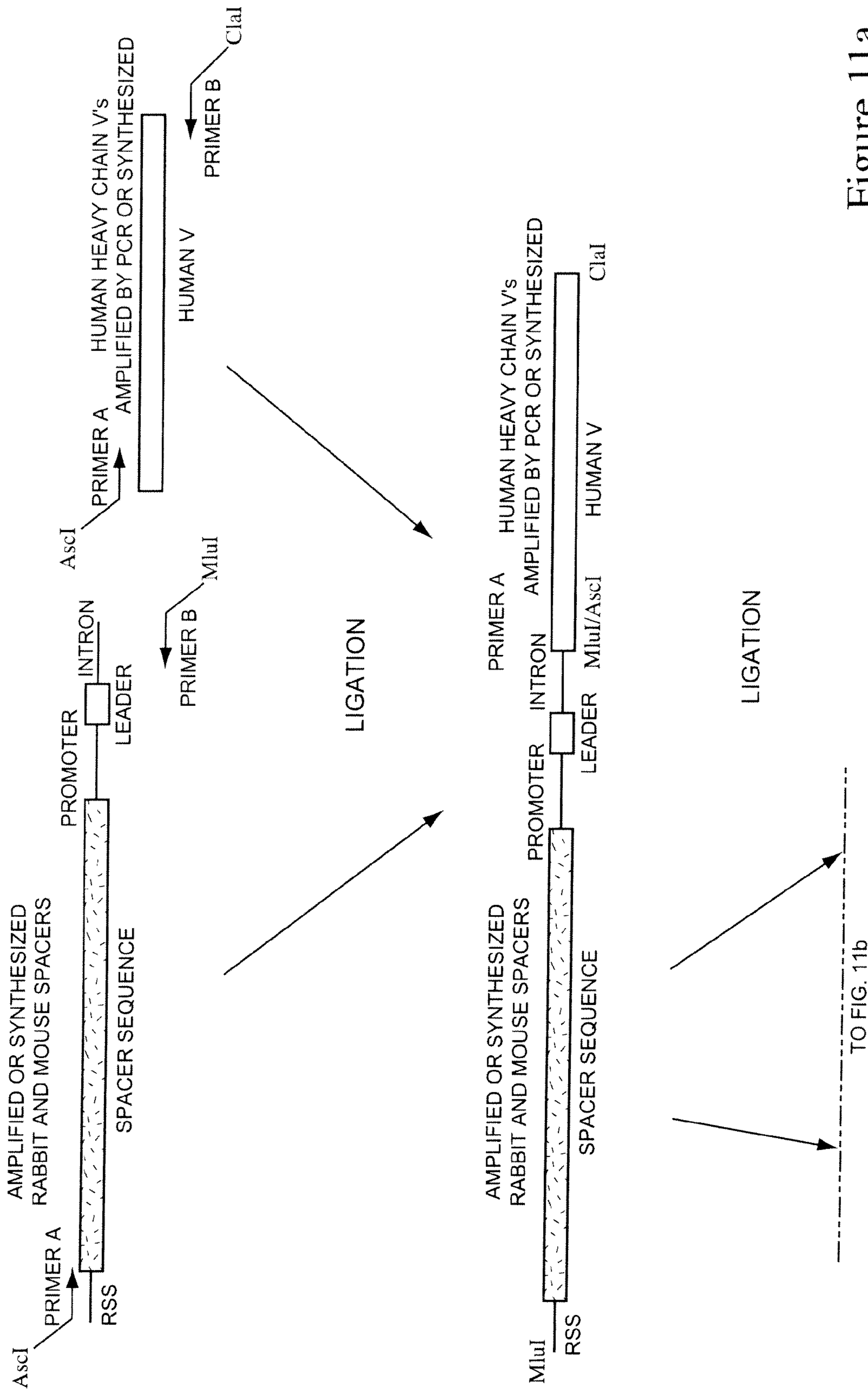


Figure 11a

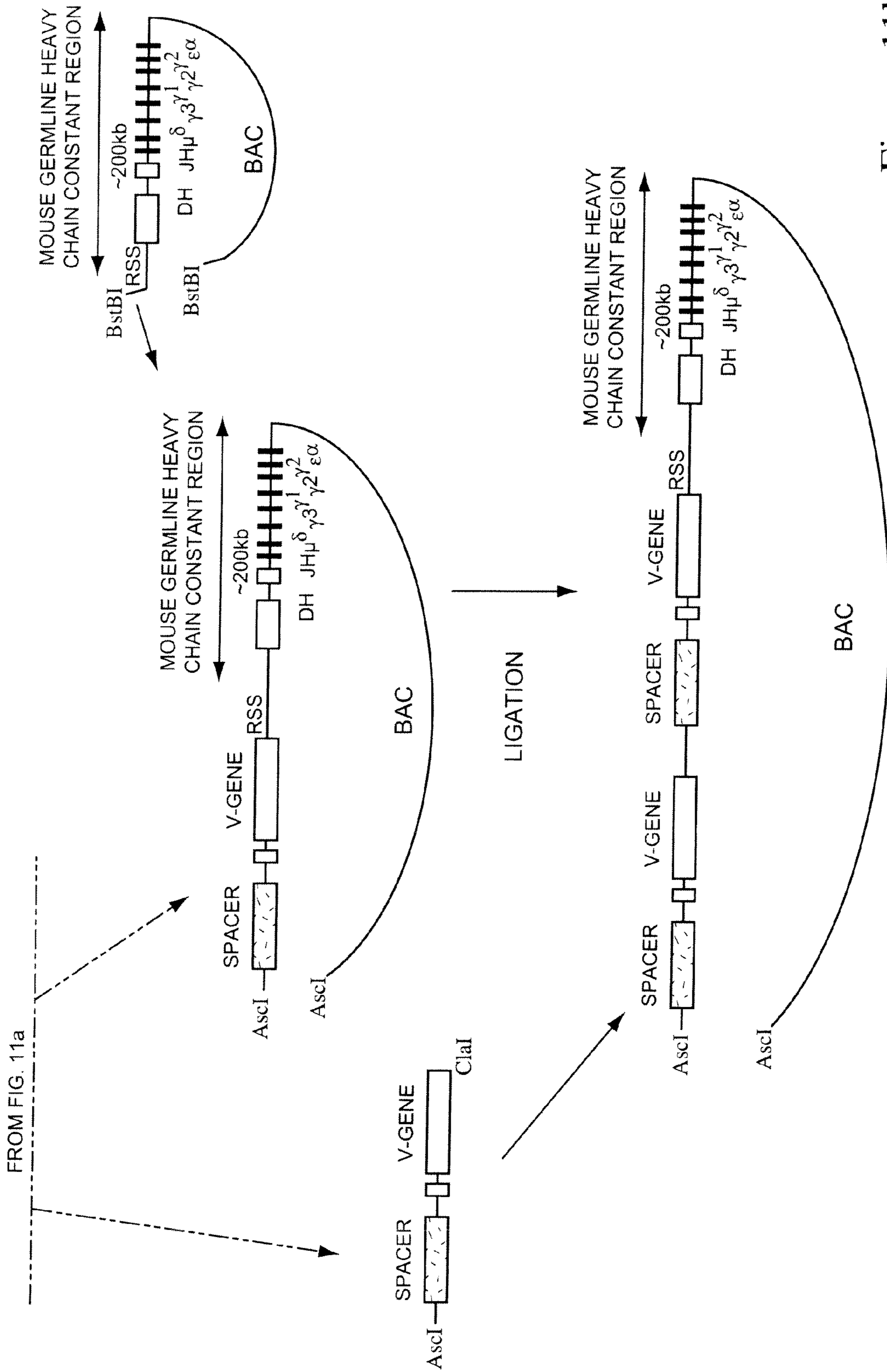


Figure 11b

Figure 12a Unit1 Seq ID 187

gcgacgcataacttcgtataatgtatgctatacgaagttatagaggatcctattaattgtttgtaattaagaggacaggaattcagt
gtcagggggctgctcttgccttccaatgtgtaccactgaagcctctctggatttatcagagatactcaatggactcttaacagcact
gtggcacctgacctctgctgccttcagcctccctcctggctgggtgtgcttgtgtgaggagctgtgggaggctggcagttctg
tgggatgaggttaaggccagctgaatagcctgggggctggcagggggcttagtgagaggagcagggctggggaatattgtga
acagcaggtggcactgctattctgaggaaggagcagcttctgcaaatgctttgcaatgcaggatgacaaagtgcttctgct
agctctaggccctgctcatggctgtgatggctgcttaacggcagagtgccagatgacatgcagatgaccagctct
ccatcctcgtcttctcttaggtgagagtcaccatcacatgccaggaaagtcaggcagggcattagccatgttttagcctgat
acaaagagaagccagggaagcctctgagctcctgatctacgatgcatccaattgcaaacctgggtcccattgcagttatgtgg
cattggatccaggacagattgattctcaccattagcatcctccagctctgaagttgctgcaactcttattattggcaacagtataaaa
gtgacctctgtacacttttggccaggggaccaagctggagatcaaactaccagcagaagacctctggcagtgcatctgtcgt
gtgctgaaaccaacagacacaccaacaccacaggacagcttctgcaagttctccatctcctggcagcttcatgacacgttaa
ccatcgtgggattcaagctgaggatgtagctgtctatccctgtggcaacagggacagcagtggttaagcctggtaggtgatac
aggagatgtcaaaaaaaaaagaaaaaccaacatgtatggggttaaaaatggaggctctctgccccttctctcatggctgagct
gggctgtgagtgaggttaagttcctgtctcgtctgcaagcactgtctgaatggctcctattgtgtgtgtggtgcatgagggatt
ctggggacagagggcattgtctgtccctataaaggccc aaagcccctggctgtgtacagtaagtccgctggggcacctgagc
tgtgggacaggtactgccaggagaggggctcaggccatgatccaataccaatggctctgtgtccccatgctaagggctatgct
gcagcccactcctcctttaaaccctcttaaactcctacagttctaatgtctctcctccaattaatcctcaccatagt
ttgatatccactttgggtcccagggccgaaagtgaacggaggggacattgtaagtccgtgaccgtaataagttgcaacatctcagg
ctgcaggctgctgatagtgagagtgaaatctgtcccagatccactgccactgaaccgagatgggactccagattgcaaatggat
gcactatagatcaggagcttaggaacttccctggttctgccgataccaatttaaataactgctaagccctgactcaccggcaa
gtgatggtgactctgtctcctacagatgcagacagggaggtggagactgggtcaactggatgtcacatctggcacgaggaga
gaggggagaaaatggagctcagtgagtgctgcacacacagccctcagcgtactgcgggtgggataatactctgagcacaca
gtcccaaatggaacctcaggactctctcatctgcttcaggatgaacaatgtgtgccagatgtgacatccagatgaccagctcca
tccttctgtctgcatctgtaggagacagagtcaccatcactgccgggcaagtcagagcattagcagctatttaaattggtatcag
cagaaaccagggaagcccctaagctcctgatctatgctgcatccagttgcaaaagtgggggtcccataaggtcagtggcagt
ggatctgggacagattcactctcaccatcagcagctctgcaactgaagatttgaacttattactgtcagtggttacagtacac
ctccgacttccggcccgggaccaaagtggatatacaaacaggcagcagtaactcctggtttgggtgatacagggagatgtcctccc
cccaaaaaaccccaacaacaaaaagcatatagggggtaaaaatggagttttctgccatctgtctcacagccaagctgg
gctgtgagctgcggcaggtcctggcctctctgcaaaagctctgtttgtgaatggccttctcctctgtacctgtgtgagggag
gagattgtggggacagaagccacttctccttgcctacaagggcacagcactgtgtcatccccagccctcctccatgttactgct
ggcagtcctcctcctgcttctgaagagacatggatgtaggtgaggtctcttctgcaacagtggtgttcacccccacagctgc
caggtgagggctgtgtgtgctggacattggctgagctccatttctcctgtcctctccaggtgccagatgtgccatccagttga
cccagctccatcctcctgtctgcatctgtaggagacagagtcaccatcactgccgggcaagtcagggcattagcagtgctta
gcctgatacagcagaaaccagggaagctcctaagctcctgatctatgatgcctccagtttgaaaagtgggggtcccataaggt
tcagcggcagtgatctgggacagattcactctcaccatcagcagcctgcagcctgaagatttgaacttattactgtcaacagt
ttaataattaccctcacacgttcggccaagggaccaaggtggaaatcaaacagtgtgcctgcaccaggggaactgggagagga
gaggggggagaaaatggagttcattgagtcctgatatacggagtcctgctaggcaatgagataagacagctgagcccacag
accatcttttacggaccagaagtgacatccagtcacccagctccatccacactgtctgcatctgtaaggagacagtgccatc
actgccgggagcagcagcattgtagtaatttagactggatcagcagaaaccagggaaggcccctaagggcctgatctat
gctgcatccagttgcaatctggggctcctcgcggttcggtagcagtgatctgggacagatttactctcaccatcagaatcctg
cagcctaaagatgtgcaacttattactgtcaacagtataaaaattaccctatgacgttcggccaagggaccaaggtggaaatcaa
aacctgtgatctatgctgacaccaacagaccaccaacaccacggggcagcttctacaatcctacacgttctggcagcttca
tgagcacattagccattgtggggactcaagctgaggatgtggctgtctatttctgtggcagcagggacagcagaggttaagcctg
gtgaggtgatacagggagatgtcaaaaaaaaaatgaacaacatgtataggagtttaaaaatggaggtttctgctctctctcaca

Figure 12a Unit1 Seq ID 187 (con't)

ggtagctgggctgtgcagtgagatgagttcctgtccccctgcaaagcactgtctgaatggctcctgctgtgtgttggtgca
tgaggagagtgaagacagagggtacttccctgtccctgagaagctgtgcatgggtgcatccccagccctgctgcatgta
cactgtctgccctgtccttctgatgccgaagctgcttccatgctgggatgctctgggctgagctctgatttccaaaatggcagtgg
tttcttctgatgggtggtattctctattctactgctgtgcaggaggctgtgtgtgtgtggctaccactgagccctgtttctct
cctctccctcaccaggaccagaagtgcacccagtcacccagtcctccatccacactgtctgcatctgtaaggagacagcgtcac
catcacttgcgggcgagtcagagcattggtagtaatttagactggtatcagcagaaaccagggaaggccctaaggccctgat
ctatgctgcatccagtttgaatctggggctccttcgcggctcgggtggcagtggtatctgggacagatttactctcaccatcagaat
cctgcagcctaaagatgttgaagttactgtcaacagtataaaaattaccctatcacttccggccctgggaccaaagtggatc
aaaccagtgagggcattgaggctgggaacaatgcagtgtgtcctttgcaggaacagggataacggggcactggaccag
aattgattactgaatgaaaagggtcagtaagagaggacatgcgtgtgatgcacagctctggcagaagggactggattctcctcag
ccctgactcaacaagggcagaaagccttggtttgaatcgcagtctgcatgtcacccccacatctcccacatcactatacgttga
tctccagcttggctcccctggctaaaatggggagggtactataactgttgacagtaataagttgcaaaatcttcaggctgcagg
ctgctgatggtagagtgtaatccgtcccagatccactgccgctgaaccttgatgggaccccactttgcaaacctggatgcataa
gatgaagagcttaggggcttttctggttttctgataccaggctaaataactgctaatgccctgactggcccagcaagtatggt
gactctgtctcctacagatgcagacagggagaatggagactgggtcatccggatggcacatctggcacactttgggacctgagc
cagactgtacctgtccctacaaaggtacagcactatgcatccccagccctgcttcatgttctgctgcaagccctgttctctctg
gagctgtctctgtctgggatgctctggtctggactctcacttctaatgtgccgggtggccatgactgaggtttctctccttfaatgt
gggtcctcccttccactgtgtacgcagaggctgtccatgtgggtattccctgagccccactttctctcctctccctctccaggt
gtcagattgacatccagatgatccagctcctctctctgtctgcatctgtaggagacagagtcagatcattgtgggcaagt
agggcattagcagtaattagcctggatctgcagaaaccagggaatcccctaagctcttctctatgatgcaaaagattgcacc
ctggggtctcagcaggttcagtgccaggggatctgggacggattcactctcaccatcatcagcctgaagcctgaagatttga
gcttattactgtaaacaggacttcagttacctccccattttagccaggggaccaagctggagatcaaacagtgccagggggag
atgagggcgtgaattgatggccagagctgaaaatgatgggttccacccttctcattatgacttttgtgagatcttctctcatgat
gtcttccctggttctctgtggttggaaaggagcagaaggcactggcaggggtcttctgggagagctggcaatagcagggatag
tggtgccccacatggaagaaaaggacacagctatggccactctgatgagaggtgtccagagcacagcagcacatccctgtg
cttcagacatctctgtgccctggggtgtggaagggaatgattcatagcatggccaggtggaggttaagagtaacatgcctactg
cagtaattctgcacatagagaaggaagaaagacagaaaatcttctacagttctaaagttctctctcttccaattaatcatcatca
ccatagtttgatatccacttgggtcccaggccgaaagctgagggtaattattaactgttgacagtaataagttgcaaaatctca
ggctgcaggctgctgatggtagagtgaaatctgtcccagatccactgccgctgaaccttgatgggaccccactttccaaactgg
aggcatcatagatcaggagcttaggagcttccctggttctgctgataccaggctaaagcactgctaatgccctgacttggccgg
caagtgatggtagctctgtctcctacagatgcagacagggaggatggagactgggtcaactggatggcacatctggcactgg
agagggagaggagagaggggagaaaatggagctcagtgatgtgtgacacacagcctcagcatgctgcagtgggactgag
caccagcactgtcagctcatttctccaggtgccagatgtgacatccagatgacccagctcctcactgtctgcatctgta
ggagacagagtcaccatcacttgcgggcgagtcagggtattagcagctggttagcctggatcagcagaaaccagagaaagc
ccctaagtcctgatctatgctgcatccagtttgaagtggtggtccatcaaggttcagcggcagtgatctgggacagattca
ctctcaccatcagcagcctgacgctgaagatttgaacttattactgccaacagtataatagttaccctcccactttggccaggg
gaccaagttggagatcaaacacagtgacacagagcaatggagatgtgatacacaacctgtcagtgacaggaacagttggca
ctggcactcactactcctggactcacaagtccagatgtaacatccagatgacccagctcctcctgcatctgtagg
agacagagtcaccatcacttgcgggcgagggcaggcattagcaattttagcctggtttcagcagaaaccagggaaagtccc
taagcactgatctatgctgcatccagtttgaagtggtggtccatcaaggttcagcggcagtggtctgggacagaattcactc
tcacaatcagcagcctgcagcctgaagatttgaacttattactgtctacagcataatagttaccctcccactttcggcggagga
ccaaggtggagatcaaacactgatgctggtagtgcaggggagatgtgaaactgaggtacagccctgaattaaaaacgtctgtt
tctgccttctcccttatgttgttctgggctgtgtcactgaggcagtttccctctgcatggccatggctttgatcatctttctgctgc
ctcagtggttgaaggtgactctccagttaccgagggaacatgaagatcattgtctctggtgtagcagtagctatggtgtg

Figure 12a Unit1 Seq ID 187 (con't)

gctgcatctggcttcaacagcaagttaaccattactcatgttgaaaccaaggatgaggctgtctaagatgtgtgagctagcatagc
agtgaggctggttagagtgacaaaggaagcatagctgtgatgtctacttctggagtgcactggggcatgaaagcgaacacagt
caaagccacaaccacacggagggaacaggaacatgtctacagccataactctgcgtggccaaaatgctgaaagtcagaaaac
cagcagggagcggggctgggtgcagtgcacccaaccttctgtggggacagctgtagaacatggaattcaatctcagtggagt
cgaaccaattggcaggctgtcagcagaggcattccccagtgtcccccttctgtaaatgtgaaattacaacaacgctgccaggca
acaaactgtattgatctgcttgagggtaggaaggccttctagacagatgaggataggcaaaaatcaatgggctgtgtcaaacca
ttgacgttcaagaacgttaagtcaccaggcctgcacttaggtcataacaacacaaggcagcagtgtaggctgaagagatggttg
attagagggttctgtagaatggttcaattctgtgatcatgaagataacttccaaacaaaattggttaggataggataaggtgctc
tggccccagaatcacctgcaacaaaaatacagcagggaggcataaacaattgcggccctgcagaggggcaggcactctg
cctcagttgatctccaccttggccccctggccgaacgccggagggttaactataaactgttgacagtaataagttgcaaatcttc
agactgcaggcagctgatggtgagagtgaatctgtccagatccactgccgctgaacctgatgggaccccactttgcaagt
ggatgcagcatagatcaggagcttagggccttccctggttttctgataaccaggctaaataactgtaataacctgactcggcc
gacaagtgatggtgactctgtctcctgtagatgcagagaatgaggatggagactgggtcatccggatggcacatctggcacctg
gagggggagaggagagaaaatggagctcagtaagtgcctgacacgcaacaccagtgccagaagatagcactgatcccaa
gacaaggagaccatgcatgccaaagaggttctcttctctcccacttctctcagtcggctctagggtctccccctctctctctg
ctctcttccacctgtacatcagatcccagggttctgtcatttctgtcctgcaggcattgcctggggccctgtggcaagaccag
ttccagatgcgacatccagatgaccagcttccatctctgtgtctgcactctgtaggagacagagtcaccatcacttctcgggcga
gtcagggtattagcagctggttagcctggtatcagcagaaccagggaagcccctaaactcctgatctatgctgcacccagtttg
caagtgggggtcccataaggttcagcggcagtggtatctgggacagattcactctcactatcagcagcctgcagcctgaagatt
ttgcaacttactatgtcaacaggctaacagtttcccccttccctggccctgggaccagggtggacatcaagcccgcagtcca
ccaccagcatatcgtttgatctactttgtcccaggggcgaagtgagaggggtcacttttatactgttgaccaataataagaagt
gcagcaactcagactggaggatgtaatggtgagaatcaaatctgtcctggatccaatgccacataactgcaatgggaccag
gtttgcaaatggatgcatcgtagatcaggagctcagaagcttccctggcttctcttctgtatcaggctaaaacatggctaatgcct
acctgacttctctggcatgtgatggtgactctcactagacaggaagacagcggagatggagactgggtcatctgcatgcatat
ctggcacctggagagggagaggagaggggagaaaacggcaatcagtggtgctgacatacacagcttccccccgcaaa
gcagcaggacagagggcccatgccatacaggcccaggctcactgaactgttggtgacctcactagtaggagaggggcca
gacctggtgctactccaacagttgtctatggacccaactcccccttttagacccccacccttctacagcacagccacctctgca
agcctggtgtccatggatactggccacccccatccatgctccccctgctcctgtagccatttctctgatctcagaagaccaatc
atggttagccactgggatccaaaatgagcacaagactatctactctcggtagcttgggaaacagcactgatactggtaga
atggcacaggagatgggaaactgagttacaaacctgagttcaaaaatggaagcttctgcacttactgcattatggctggacagg
gctgcaggggctgagtcctgttctgtcctctctgcacggccctgactgtaaatggcctcctctctcttaccacaatgtacctgt
ccctgccaggcacagcactacgtcaaccctagccctgctccatgttctgtctgtgagctcctatctctcttagagctgccttctg
gctgagatgctctgtgtggctcttggccacaaacatacctatgtctgtgactgaggacccttctctctgatggtagtgttcccc
tgcggttggggagggcagggcaaatgggcacctgttagccccattactccccctctccctatgcagggtgccagatgtccatc
cagatgaccagcttccatctccccgtctgcatctgtaggagacagagtcaccatcacttggcgggcaagtgcaggcattagaa
atgatttaggctggtatcagcagaaaccagggaagcccctaaactcctgatctatgctgcacccagtttcaaaatggggtccc
atcaaggtcagcggcagtggtatctggcacagattcactctcaccatcagcagcctgcagcctgaagattttgcaacttactg
tctacaagattacaattacctccaacgtccggccaagggaacaaaggtggaatacaaacgtatggtgatagggggcagatgtgg
ggggaaaaaaaaaaaaaaaaaagacacatataggggataaaaatggaggtttctgcccactctgtctcacagccaagctgggct
gtgcagctgaggcaggttctgccccctctgaaagcactcgttgaatggtctcccccttctgtacagtgcagtgcaggaagaga
taccaggtatgtccagacctgttctgttactgtggcagacctatctctctgttgaagaggcatggatgtctgtgaggcctcat
ctgccagggtggtgttctcatcccacctgccagggtgagggtgtgcgtgtcaggcaaacactgagccccatttctccccctct
ccccctccagataccagatgtgacatccagatgaccagcttccatctccccctgtctgcatctgtaggagacagagtcaccatcac
ttgccgggcgagtcagggcattagcaattttagcctggtatcagcagaaaccagggaagttcctaagctcctgatctatgctg

Figure 12a Unit1 Seq ID 187 (con't)

catccactttgcaatcaggggtcccctctcggttcagtggcagtggatctgggacagattcactctcaccatcagcagcctgcag
cctgaagatgltgcaacttattactgtcaaaagtataaacagtgccctctcactttcggcggaggaccaaggtggtgatcagtcc
cacagtgactcagatgtaatacaciaaacctcccggcagtgaaggagcagataataccttaactgtctgtcttacagggtccacc
tccatcaatacccctatgctgcagggctcaggtcctcttaggtgccaggctgcaccctgctggctcccctcccactaccctacac
ccaccctttgcagtgtcccctgcatgcaactggggcaaacacttcctcacctgtccgctcccctgctggagggccacagccacctggt
tgatacatcagcatagcaccagttcttcccgaagctggccctcccctggactctctactgagccccattttctcccctctccttcca
ggtgccagatgtgccatccagttgaccagctctccatcctcctgtctgcatctgtaggagacagagtcaccatcacttgccggg
caagtcagggcattagcagtgccttagcctggatcagcagaaaccagggaaagctcctaagctcctgatctatgatgcctccag
tttgaaaagtgggtcccataaggttcagcggcagtggatctgggacagattcactctcaccatcagcagcctgcagcctgaa
gattttgcaacttattactgtcaacagtttaatagttaccctcacacctcggccaaggacacgactggagattaacccatggtg
acacacagcagtgaggaagcagatgcaciaaacctcctgacagtgaaggagcagccggc

Figure 12b Unit2 Seq Id 188

gccggcaggtttgctgtctctgttgatggtggcacaggacctcaccacagccctacctcactgtcctgcaatatgggtggctgt
gcacggtgtcctgaacacgtcccaacaagatgcacagcac.gcatcaggcccagcccctgcctgtagcagctgatgccctgtc
cagcttctgcctgagctgcctctgtgctgggatgctccatgctgagctctgatcctccaaagtgcccatagctgtggctgggatcc
cttctgctctgatggtgggtggcacattgcaggaacagagcccagaccagaacagctcagtacagagccagctccagcaggga
gagaatggggctgccagcagcaatgtggaacagctggggatggcacagagctgtgcctttcagggacaggtacagtgtgact
caglacccagagtaacctctcatacaactacatataccaagggaaggcatttacaagcatggccatgcagagaagatataaatc
ctcagctcacagacctgctcagccataaggcatcaagggtgaaaaacctctatttttttaacctgatcaatccatcagttctc
acctcccgtgaggcaatacgggtggcgtccacctggccctggccgaacgtcggatggctgctgtaagtctatagacagca
ataagttataacatcttcagggtgcaggctcctgatggtgagaatgtgtgaaatgtgtcccagacctactgccactgaaccgtgac
gggaccccagattgcaaactggatgcagcatagatcaggagcttaggaggattctctagtttctactgatactagcttaataattg
caaatgccctgactgccagcaagtgatggtgactctctctctacagatgcagtcaggaggatggagactgagtcactctgg
aaattacatctgactcctggagagggagagaaaaacagggtctgtgtgtctgacacgcacagccctctgtttggcagtgagg
tgaggctgctgagcacacagacacacatgggacagcaggagaccacatctgctccaggattgacatctatggctggtagg
cagaagatacctggcaatgccccatcactgggatccatgtaacactgtagaccacagggcttctcttaccattctctggggtc
caatccaggcctacacacatgaactgtcacactaactaccactgtggtctaaactgtggagaactgccattactgcgggtggc
aatgacaggagccaaggcctgaggatggcacttatcaccaggatagcaggacaagggttcacaagaaaaaagaaagggttc
ttttctctcccgtctctctcagctcagctctgtggtctcttctctctctactgctctctcaccctgtacatcagatcccagggt
tcgctgcattctgctcctgcaggcattgcctggggccctgtggcaagaccagtgccagatgtgacatccagatgaccagctc
catcctcactgtctgcactgtaggagacagagtcaccatcactgtcgggcgagtcagggcattagcaattattagcctggttc
agcagaaaccagggaagcccctaaagtcctgatctatgctgcatccagttgcaaaagtgggtcccatcaagggtcagcggca
gtggatctgggacagattcactctcaccatcagcagcctgcagcctgaagatttgcaacttactgccaacagtataatagtta
ccctctcactttcggcggagggaaccaaggtggagattaaagtatttgacaatgtgtagtaggaacataaacacagccacattc
agtaaaagtttcaggccaccttctgtccccgagctttgggaattgtgacttatgttactgtttgggaattcaggaggcctgtcct
atctgaatggccaagacctgactgttaccacagatcatattccaggaatgagggtgatactgggtatagacccatcactcctgtc
cctctccaggagtcagatgtgattccagatgactcagctccatcctcctgactgcatctgtaggagagagagtcaccatcact
gctgggcgagtcaggcattgcaattatfaagctagtatcagtagaaactagagaatcctcctaagctcctgatctatgctgcat
ccagtttgcaatctggggctccgtcacgggtcagtggcagtaggtctgggacacatttcacacatttcaccatcaggagcctgca
acctgaagatgttataacttactgtctatagacttacagcagccatcccactttggccaggggaccaagctggagatcaaaact
atggtgacaatgattaaaaggaactgaattaataaattataagcctgtttctgccatctcctttggccatgcaagactgtggcca
tagcagtttctgttccatctgcatggccatgctatgaatgactccctccacaccgagggcacaggagatgtctgaagcacag
ggatgtgctgctgtgctctggacacctctcatcagaagtggccatagctgtgcccctttctccatgtggggcatccactatcctgc
tattgccagcactcccagcaagaccctgccagtgcctctgctcttcttaaccacagcagtgagggtgctgccccagagctgctc
ctctggatgctgtgccccacatttctgaacacacactgccatccagcaatgtgggacagggaacaccacagcaggaagaga
cctcaatcacagccatagtgagggcagacagaaacctgtcatttttagctctagacatcacatcactcccatatctcctgtgtctc
gtacctacaaaactgctgtcataggccccactaggacaggaacatgaatggggctctgcaaaggcagcggccacagcagctgtg
gacccagagagcagaactgactgcctcaacagctccgccatgcaggagatgtgcagcaacaaggaggggaggaatc
caacagtccgccaccagtttgagatcagcttggctccctccgcaaaagtgagagggttaactattatgctgtagacagtaataa
gttgcaaaatcttcaggctgcaggctgctgattgtgagagtgaattctgtcccagatccactgccgctgaaccttgatgggacccc
actttgcaaaactggatgcagcatagatcaggcgttaggggcttccctggtttctgctgataccagcctaaatcatttctaatgcc
tgacttggccggcaagtgatggtgactctgtctctacagatgcagacaggaggatggagactgggtcatctggatgtcacac
ctggcacctggagagggagaggagagaaaaatggggctcagtcctgacacacactgccctcctcctcctcctcatggggagg
agaccccagagcacacaagcacacacagaatattcaaacaccgacttctaatccatgcttaacagcaactatgactggtacaag
cagaagactcctgacaataccactggcactttgtaatacctccacaataagccccctctcctcctcctcctcaggtgccagatg
tgacatccagatgaccagctcctcctcctgctgcatctgtaggagacagagtcaccatcacttggcaggcgagtcaggac

Figure 12b Unit2 Seq Id 188 (con't)

attagcaactatttaaattggtatcagcagaaaccagggaagcccctaagctcctgatctacgatgcatccaatttggaaacagg
ggtcccatcaagggtcagtggaagtggatctgggacagattttactttaccatcagcagcctgcagcctgaagatattgcaacat
attactgtcaacagtatgataatctccctctcactttcggcggcgggaccaaggtggatctcaaacttagtgacgggggggagat
gtgaaactctclacagccctgaattaaaacatggatgtttctgcctttctccctcaggatcacgctgggctctgtcactcaggcagg
ttccctctgcatggccacggctttgaacatctttcctgttctgecteattgtataggactctcccagctacctggaaacacaaaaata
ccagatgtgacatccagatgaccagctctccatctcctgtctgcatctgtaggagacagagtcaccatcacttggcgggcgag
tcagggcattagcaattattagcctggatcagcagaaaccagggaagttcctaagctcctgatctatgctgcatccgctttgca
atcagggggtccatctcgggtcagtgagcagtgatctgggacagatttactctcaccatcagcagcctgcagcctgaagatgt
tgcaacttattactgtcaaaagtataacagtgcccctcccactttggccaggggaccaacctggagaccaggcaaatgcagac
aacagttgtacagtlactgcttcccactgacacagagcagcaggtacaactgatgactaacctcctgccagtgaggagcagc
tggtctttttgctggctctgtttgatggcgggtgctggttctaccatggcccttccctcattgccctggactacatgtggcagggca
aaaatcacatcccagcactgacagcagggaaacctgtggaccgctgagcagccaggccaaagctccctacttagaggggatg
ttcccatggcacaaggctgggctggctgtgtttctcttctggcacgagggcagccacagaatcttcagatcaggatgattgtgt
gatctgctcctacctcagctcaactggcatcacctacagcacagtcccaggcctgtgtgcaggtggctgtcagagatctccaag
gaggagactccatgatctctccgggcagcctggatcagtgctacattacctgcggagtaaggatgtgactcctgactctggccag
acagaacctctgcttcagctctgtccactgctcctgtactgggtgctgaatgctgctggaaaaagcctggctctgttactttgca
ccctccttcaggtagcgttaaggagagagagaaaatagggtcagcaattgcccaacatgcacagccctcaccaggctgt
cagatgaggcacgcaattccacaaggacacatggggtagcaggataccgttccagatgcgacatccagatgaccagctcca
tcttccgtgtctgcatctgtaggagacagagtcaccatcacttctcgggcgagtcagggtattagcagctggttagcctggatca
gcagaaaccagggaagcccctaagctcctgatctatgctgcatccagtttgcaaaagtggggtccatcaagggtcagcggcag
tgatctgggacagatttactctcaccatcagcagcctgcagcctgaagattttgcaacttactattgtcaacaggctaacagttc
cctctcactttcggcggcgggaccagggtcagctccgaccatggtgacacacagcagtgaggaaagtgatgcacaaacctcct
gtccgtgcaaggagcagctggcaggtttgctgtctctgtttgatggtggcacaggacccaccacagccctaccttactgacct
gcaacatgggtggctgtgcacgggtgctctgaacacgtcccaacaagatgcacagcactgcatcaggcccagcccctgctgta
gcagctgatgccctgtccaactctgccagagctgctctgtgctgggatgctccatgctgagctctgatctccaaagtgccat
agctgtggctgggatcccttctgctctgatggtggtgacacattgcaggaacagagcccagaccagaacatcttagtaaggagg
cagctccagcagcagcagaacggggctgccagcagcagggaatgatgctgtgccacagtaggaacagggaccaagaggat
cacttcccacgcaataaagtataaaagagagggcattcaaaaacacagccatgcagagcagagagaaaacctgactcagcccc
acagctcctgctcagccttaagtcagcaagtgcagaaaacctccatttcaactcaggtctgtaactcagttccacatcctctttgtctc
tataccagcattaagtgtctatcccagccaccacagcctcatcttctgacctagtgacaactaaccacaattggtcttccagggc
ccagggaatagctgactgggacaggagctgccagtgaccatgaggttctgcatgagtgggctgtgttgggagttcagg
gtataaagaaaggagttggagtccatggcatagaccattggcataaggatgtgcatctgctgggatcacaccatggcctgtgcc
cctctcctgctggcagtgctgaccacagctcaggtgccccaggaacaccggtgtgccagcctttgggactgggccccatc
cccctgctttttaagacttggcatcaattcaccacaacacctctctgcagcaccagtttgatgtccagcttgggtccccgggcaaa
agactccggagggttaactattaagctgttgacagtaataagttgcaaaatcttcaggctgcaggctgctgattgtgagagtgaattc
tgtccagatccactgccgctgaaccttgatgggacccactttgcaaaagtggatgcagcatagatcaggagcttaggggcttctc
cctggttttgctgataccaggctaaataactgctaatgccctgactggcccggcaagtgatggtgactctgtctcctacagatgca
gacaggaaggatggagactgggtcaactggatgacacatctggcacctggagaaggagagaagagaaaatgggctcaacaa
gagcctgacacacagccctcccagcagcagtggtggggaacagcaccattaaaggaaaaggacttcagccatagc
cacatcaaaggatgagagcccagcccagagcagcccagcacagaggcagctccatggagcagggctggggatgacaagat
gctgtatctttacaaggataggtacagtggtgtaagtgtcagagtaatcttcatacatctaggtacagacaggaggcattaca
aacacggccacgcagagaggacagaaacctgcctcagccccacagcgtgataagccattactcagtaagtgcagaaagttc
catttttaactcaggcctgttactcagtttccatctcctgtgtcactgatccccaaacttggactccagtgaggtaacctgatca
gccttctgtgagctgagaaaaggagtgtagtgagcagggagagcactgcaataggagctgatagtgatcacacaggcctgtac

Figure 12b Unit2 Seq Id 188 (con't)

tatgggcagatgggagtttaagagtgaattgcggccatggcatatgcctgtcagcatagggatgctggaggaattgcacctgg
catgagccccctcctcctggtgatgcttcccacagctctgacgtcctgggaaggctcactgcaccttgtgtcagtgaaactgc
aataagctatcaagattgatataatttgcctcctgatctactgtgagaccacacctggagtactgcatccagtctggtaccac
caacacaaaaaggacatggagctgctggagcagggccagaggaggccatgaagatgaacagagggctgaagcacctccc
ctatggggacaggctgagagacatggggctttgagactggagaagagaaggctccatgggaccttcagtgtcctccaact
tgaagggtccttgaggaaagctggggagggacttctataaggacatatagcaacaggacaaaaggaaagggtttaaactg
gaagaaagtagatttagctagatagtaacaagaaattctttacagtaaggatcacgagacactgaaacaggttgcctaaagcag
ctgtggatgccccctccctggaagtgtccaggccaggctggatggggctttgagcaacctgttctagtgagaggtgacctgcc
tacagcaggggagttgaacctagatggtcttaagggtccctccaacccaaatcattctatggcaattctatagagaacacctatgc
tgagagtagctacaagtatggctggtgtgccagatgtgtcatctggatgacctgtcctcctactctctgcatctacaggag
acagagtcaccatcagttgtcggatgagtcagggcattagcattatagcctggtatcagcaaaaaccagggaagccccctga
gctcctgatctatgctgcatccacttgcgaagtggggctccatcaaggttcagtggcagtgatctgggacagatttcactctcac
catcagttgctgcagctgaagatttgcacttactgtcaacagattatagttccctccgacttccggccctgggaccaaag
tgatatacgaactcacagtgacatggagcaataaaaatgatgcataacctttgcacatctgtaagctataaggtgagagaaagt
aagcaataatagcagagcagcacaatcctaggctacagcaaatgagaacaagcccagatgcagccacagaaataaagaaaa
aaaactgcttaccttagaaagcctcatatagacagggatctccagcaagataccacacctccactgccaaccttaaatgagg
gctgggatgggctggatcctggctccaccttcaactcaggtacattgcacacacctgagctcccctggctggccctgcc
tcccaccaggctcaatcactgcttgggctgtgactcagcatctcactacagcatcacaggagcgggtgtcggctctgct
gttctatgtgggtgccccgggacacctgaaggcatgagtgccagtgagggtcccagctctatccatggtgaatttgatcca
ccttggctcccaccgccgaaagtgagagaataactattatactgttggcagtaataagttgcaaaaatcatcaggctgcaggctgctg
atggtgagagtgaattctgtcccagatccactgccgctgaacctgatgggacccacttccaaactggagggcatcatagatca
ggagcttaggggcttccctggttctgctgataccaggccaaccagctactaatactctgactggccccgcaagtgatggtgact
ctgtctctacagatgcagacaggggtggaaggagactggtcatctggatgtcacatttggcacctggagagggagaggagag
aggggagaaaatggatctgagcaattgcctgacacacacagccctcccagactgtgggatgaggccctgtcccgcagtca
catgtggaatatcaagacacacacatctatgacaatcacaatctgattatcaaccggaccagaagtgacatccagtcaccagctc
ccatccacactgtctgcatctgtaaggagacagcgtcaccatcacttccggggcagtcagagcattggtagtaatttagactggt
atcagcagaaaccagggaaggccccctaaaggccctgatctatgctgcatccagttgcaatctggggctcctcgcggctcgggtg
gcagtggatctgggacagatttactctcaccatcagaatcctgcagcctaaagatgttgcacttattactgtcaacagtataaaaa
ttaccctatcaccttggccaggggacacgactggagattaacagctgctcctcctgggccccatctgctgagagtgatttca
gagcagccaggctgaaggctcctgcaggttttgggaaggggccagggtgactcctctgaggccccctccttccctcctcctg
gcccagcagtagacactttgggtaaccttccgtgtctgctgagatgcagtcagataggagaattctctgatccctaccacctgc
acacaggggcaagtgtcccagggtgccacatcagaccagggtgcacaggtctcctacagagaatgtctctctggggctcagc
agagagaggaccagtgtgttagaggtccacgctcctcactcagggctcagcccagcttctgctgctgctccacctgtgacagg
ggctgcagggcttgcacagcccagaacctggagaagctgagcagctcctcagtggttctggtggacgtcactgaggccag
ggcggcttctgcttcttattcctaatggccttgaacataaaagagcaataaatagaaaacatagtgagggtatgattataa
aactggctgtgcttggatcctgccacacactgggagctctacatggagtttctgctctggactgtggcctgacctgacctcagct
gacaggctcatccgggaaactaccagcagctggaggatttctctctctcttctctctcacttcttcttaccatgctcagtc
ttcaagtaataaataaataaataattttaaaaatgggaaatattacatttaataaataaataatggggggcacatgtgcaatcgtgca
gtttcaatctgatggagtgttctgattcaatgcaaaaatcaagtagaacattctcatccaattgcaactaatgacagcttactgtgg
cacataaaggcacacaaagaatagtagcacaattcttaggcctgaaataattggaagaagtcgaacagagcccttatgtcata
gatcttcatcttagtggccgggcagatgagaaacaaccaatgacgttccaactgtgctcccactgtgtccatgtgatgtgcctc
ccatggagcagggccagtgtcaggacaatggggctgtgggtgtgcaggtgcatgtgtgctccttctgtctccacctagacag
ggcagagggcagaggcagaggaggggaatgtatcgggcaactccacatctgtgttctgaaaaataaactcattgtactctcg
gtgatggtcgggtagacaaaactgctcctgacaaaagatttaagattctcatgtccctgtagcataaagtcagtcagcacacaggct

Figure 12b Unit2 Seq Id 188 (con't)

agagggaataatcagtgccagacactggagataaaaaaatggagcccagitttagtgcgcattagcagggaaatgtgatcaga
agtggagcagtgggcttcaatccagtgtctgatgtaggctgctggcttgaagtggaacttaatccatgtaccatgatgccagt
ctacctcctctctagatctttagagataaagtgaaggcttcaggagagaggagagaaggaagttatcttctgatgtctcagac
ctatggtgaggccttgaactttcacttttcttctcctgcaccttgactcagaactctggctgcaggtgggagagcacacatgtgt
gatctctcatctaaacactgtgttatecttctgagtcctggctctggcagccgcttgttctgaggatgtttattctgagtcagtacag
aggtaagtgtgggtagaagtctctctcagagacaatctccctggtgcagcggctccatgaggggacaagatgagggatca
aaggacaaacaggtgaaagacatggacctcagctttaaggtgtaactaagtattccctagaaaagcatttctactaaagaattcca
ccataagcatgaacaaattacacttctgtcctccagttcaattatagggttggcaaatgtccctcaggggaatttgggaggtgatgt
ctcctggtggaagcatgcgcactgcaggtaaacttgcagccctggtctgagctggggcagctggagacacagcccctggac
tgagttctgaactgccctgggcccctcagctgggcacagcccctgccccgcccctgctcattgcatgtcccagagcaccgccc
acctctctgggcatttaggagcaggctgtcccgccccatgcaggagcagtgccaggcaggaccagcatggacatgaggg
tccccgctcagctcctggggctcctgtactctggctccgaggtactcgttgcgcccggctcggggactgtgggcacggggctct
gtcccattgtgcgcgggcagggtgtgcgtgcggggcgtcactgattgccgtttctcccctctctcctctccctctccaggtg
ccagatgtgacatccagatgaccagctcctcctccctgtctgcactctgtaggagacagagtcaccatcacttgcgggcaag
tcagagcattagcagctattaaattggtatcagcagaaccagggaagcccctaagctcctgatctatgctgatccagtttga
aagtggggtcccatcaagggtcagtggcagtgatctgggacagatttactctcaccatcagcagctctgcaacctgaagatttg
caacttactactgtcaacagagttacagtaccctctcactttcggcggaggaccagggtggagattaaagtaagtggccgtt
cactgattcctcaccgtttctgcctgattggttgttttccacttttctgctgttgtgtcttggctggaggtcagggttctaac
gaatttcttccaagatgaggcacagaggagggaactgtctgagatttccaagtcaaaataactccccggggagagggcttg
ccgcttgtcagggaggggtttgtggagggagaaggtaaaggagccaccgtgaagttcctattccgaagttcctattctctaga
aagtataggaacttccccttctgtccaactgtgaggtcttggccctggatcacctgggcaagtttgtgatgttccagttaatgag
ccattcctggcgacccccaaaagtaaatagaagaaaataatcaaaagcggagaagacagaaatttgggggttgggagaatagg
agagaaacagcagaagtttctctacagttgccgtgtggtgggactggccgcaaaggaggttttgtgaggggtgggatgacag
agtgacttacttttggctcagggagggcgcgcc

Figure 12c

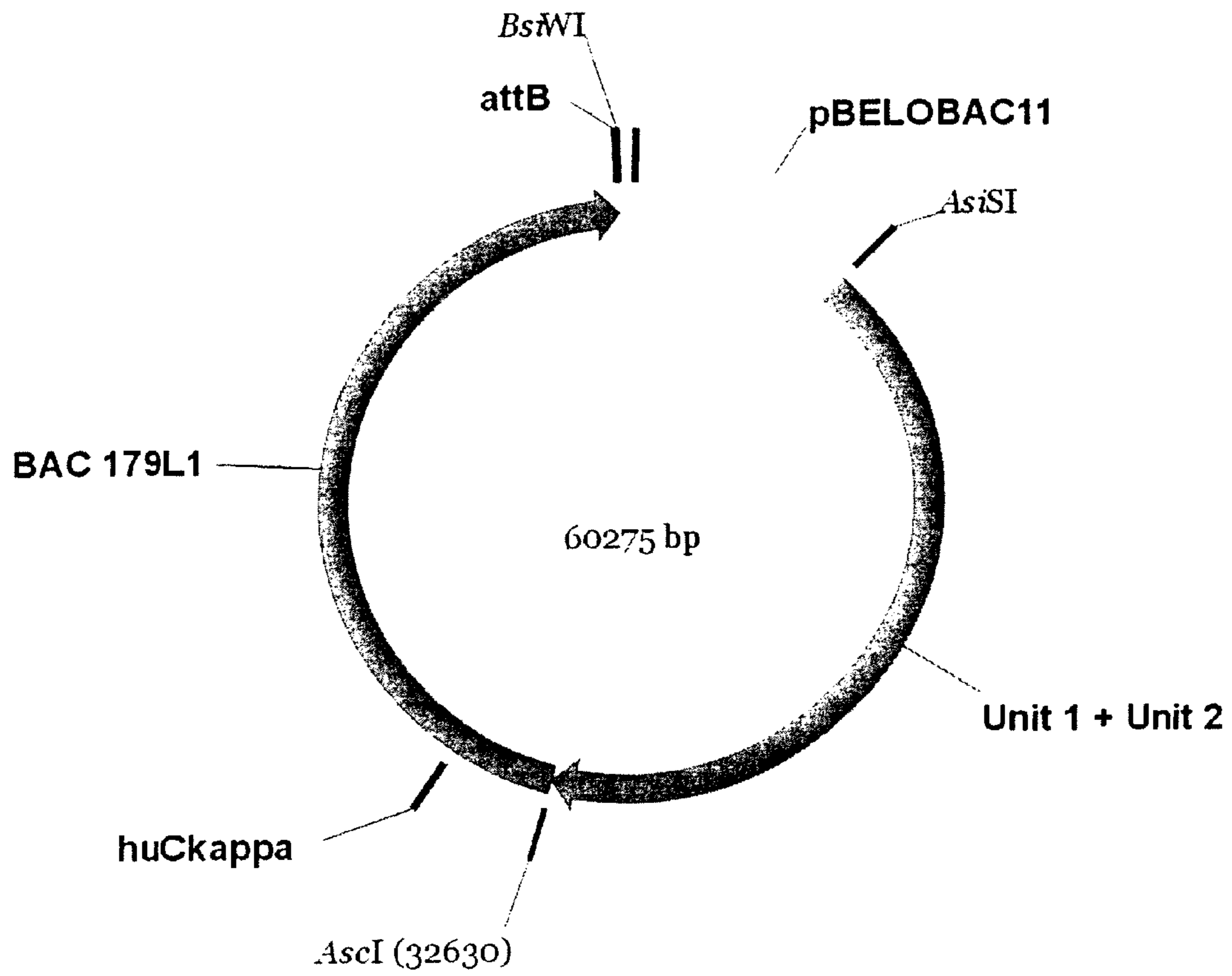


Figure 13a Unit 1 6607bp Seq ID 189

ggcgcgccataacttcgtatagcatacattatacgaacggtaaggggagtgccggctccaccagggggcgcgcaagacacact
gagtcagattctggctcgtcaggcacagaggagatgaagccctggttcctgtcaggattgggttcctctcctacagttt
gaggaagacttctactcaggattctgtcccttactgacgccactgacgtagagaagcttatgtgacaggaagaagtctaa
atggacacattcaggttgtgaacgatgacagtggcaccagggtcagagatgtgagacaatcaggggacctcgtgagtgtg
cagtctgactcaggacagcgtccaggacactgccgactataagacaatttagtttcctcaaaccagacagaccgagcca
gagtcttgattgctacctcagtttataagtcgtccatcctcctgtcagagcagcctgtgtgctgactgaaccaaccgtctat
aactgtgggtgttctgcattcacttctacagactttataccaatctccatttcccttaactcctgaaacagctctgtgtgc
acttcagctgatctggttctgctccagtgtcgtgggaaccctgcacctgctgtggtcagggagagcagaaagtgggacaca
gagcggctgtgactctggggctggagtccacatcacggggagctgtgtctgtggctccagcatgatgccagtgctcagg
ctgaatcacagctgcaggaagagccagctccagagaccatgtgtccagagctcctgtcttactccatgtctatgctgactggg
gtcatcaatccattgtgttcttcatcagtattagagaggagacaccaggctgcatatcacattgctggatggagccattgca
cgtttccatactgtactgacagtgcacctggaagacagtacatgatgatgatgaggccccatcaccaaggtgggaaac
actgatgcagttcatgctcctgggtccagctgacaagcttgagtccactgcctttatctatctccataaaacatggccaacac
aacaccagattcaaatcaatcgtcatgattggccatcgttgctatttcatattcctaaatgttgataaataataggatagctc
tatgaggtacaatgatgtgtggacaaaatgaatgcatattgagtggaaatgaagtcaggtgatgacacattcataccaaa
cacatgttttggaggagttcacctgtctcctgtcttaagagattccactcacaatattcttaacctctgtcaccatgctgtcagt
agatccctcagtggtggagcctgtgggactaaaactctgacctgctccagcgtctcctctgttccctcctcgtcagacttggc
ttaccattgtacttctgcctctgtcctcagcctttatcccatgtaggtagcaccatattatgtgttctcctggcctggatgctta
gcagagtgtactttactgacatattatcttaggcacattattctccgggatcatcaagttgtccaaaatcacagaatttctactctgt
actgctttaaacttctttaaaatgtaacttgcatttcttccatttcttctcctcatgagatgctactggatcatatgtgtgaa
ctctttgaaatggcgggtgctttaaataaagaaggaactgcagaggtgttggcacagtactgcagatcccgtggacacctcctg
ggattcatattcattgtaccatgcaatggtgcttgaagatcatgtaataattctgttctagttttgatgctttccataatggtttacta
attaacctgacacagactgtaccaggttatctctctctaccctccataaaacatgtcagctctggtatgtctgataatagcttc
taacaggaggatgggatgtctcattgtgcttctaacatggacatacatgatggtcctagattttagcattttatgtttgtcttata
tcttctttgaaattatagatgtaacataaattttaaattatcattttattggccagcgcagtggtcactaggctaactcctccactgc
ggtagactgcaaccagtttctagtctcggcggcggcggattctgtcccgggtgccccctctctaggccagctctctactgtggcc
cgggagtgagtgaggatggcccaagagctgtgacctgcacccatgggagaccaggagaagcacctgactcctggctc
ggatcagcgtggtgcaccggttacagcggccattggagggtgaaccaacggtaaaggaagacatttctctgtctctctctgt
cactgtccactctgcttgcaaaaaagtgtaccattttttaaatttgtctttatattgtccaaaaccacagagagactgagact
gacagtgatcttcaattcctggtcacttctcagatggctaccaagattcagctgggtcagcagccaggtgcttgaattct
gtccagggctcccacgtgggactacttgaacttccatggcctccagaggggcttaggaaggaatatgagactggaacc
aaactaggacacagcactcagcactcctacagagcaggaatccatcaggctcactccagtgctgctcctgtcaccacctat
catcactgcatttaaggaattgatttctccttgggattcagttgttgagatcttcaacattaggaaataggcttcaatcatatgata
attaacttcaatttatttaagatctattttatttagagacagtggtatagcaagagatgaagagaccaacagatggagagagaag
cttctgtccagtggttcacttctgtgaatggctgcaaggctatgatggctgagcctaaagccaggagcctggaactcctccagtt
cccacagggtggaaggggacaaacactttgtcatggtcagcagtgatcaccattgcaatgcctacatctgtgagttcaattgtg
gactgcatatacaataatgtcacatactatgtgtccacatctacctggatcatttctttagtatgtatgctccaggctgacctatgtt
cctactgacagaacgacctccttctaactgaataatattccatgtccaatgtgaaagaatccattcactgttctgtgaacacttg
cttaattgtagactgaactattatgaacacagctgcattacctgaacccaggactgcgggtgaattgtgacacagtacttctacc
agtgttgagctgaactcagagtcattgacagaacagggaagagggggaacacagggcagctgataacaggacgcaag
caccactcacaggaaggaataattctgcctacaccacagtggtgactcagttcaccacaacctatgacaggtccacaata
actagaactgaggtctccaggcctcccaacagagtgatggaaaggaggtggaaatgtgcatgacactgagttcatctgtgccca
ttctaccatgcacattcaatagcacacaaacccactaccatgtacagtggtctgtgtcaggaacagttttaaactaaagcaatg

Figure 13a Unit 1 6607bp Seq ID 189 (con't)

gatgaataaatgaacaacacatagttaaccttaaaaaatcaacttgcagtccttcatctattttaaagtgggcagcaggcattagga
caaaattggctaagtcccactagggtcaccctgttccc atcagagaactgactgagcccagcctcacggcttcccacactttc
cctcataatgcatcatgggaaactgtagatgacactacaggc aaaggagcccctaccaccgacgttgaattacagacacagttt
cttgcctcagggttcagaaagactaactcctaggggtcataggcatttgggatgtctagggtctcaacccaaaggcagaaatctc
ccattgtcacctttctgtccatctctacatttctgtaactctcacccttctatctctctctgtcccattgtcttcatatagatgataag
gaactaatgggatitaaataaatgaggaacatgatattgtaacactggatattaagggtgcacacatattaaaatgaacaaggta
tctgccttcaactttttaaataattataacataaaaattcatatgatctgaatcatatcacagccatcaccgtacacccccagggtcac
cacatctgccctgggcgctgtcctgtctgaggcgtctgaccccatgctgtatataggggcagctcatgcaaatggggcctccc
tgtgccatgaaaaccagcccagccctcaccctgcagctctggcacaggagctccagccccaggactcccagggtgtccactca
gtgatcgcactcaacacagacgctcaccatggagactgggctgcgctggcttctcctggctgctgtgctcaaaggtaatgatgg
ggaacgcgggacactgagctgggagaggatgtgagtgtgagagacacagagagtgtgagtgtgacagtgtcctgacatgtcgtct
gtgttgcagggtgtccagtgtgagggtgcagctgttggagtctgggggaggcttggtagcagcctggggggtccctgagactctct
gtgcagcctctggattacccttagcagctatgcatgagctgggtccgccaggctccagggaaaggggctggagtgggtctcag
ctattagtggtagtggtagcactacgcagactccgtgaaggccgggtccacatctccagagacaattccaagaacac
gctgtatctgcaaatgaacagcctgagagccgaggacacggcctatattactgtgcgaaagacacagtgaggggcccctcagg
ctgagcccagacacaaacctccctgcagggtcgcgcggctctaccagggggcgtcaagacgcactgagtgcagattctggt
cctgcacagcgcagaagatgaagccctggtctcctgtgagggaattgggggttctctctctgtagcagttttaggggaagaatt
ctactttttagattctgtccttcccactcactgacggagagaaaagttgatgtaaaaggaagaagtcctaattgaacaatttggttcat
ggacagcactgtgctcaccagggtcagagatgtgggaatattgaggactctattgagtcttccaacctgactcaggacagca
ccctcagggcactgaaagcttggacagttttagtttccctgtataaccagggacagctgagacagggctcgttcatctgttaaac
agcaactattcactcatccctctcctgccacaaccagcaagtgtgactaaaccaacctttagctctacatgtgggtgatccacac
tcactttctgcagacttaacccgacctgcatctcccttaactcctggaacagctctgtggctccttgtgtaacgtcacagcactcagga
atgactgcagctcagctgtgtctcaggctgagcttctgcaggcacctgggaagcctacagggacttctcctctaggtcctg
ggacctcctggggggctccctgcctgaactcaggagggaatgccctggacatcagcagtcagggtcactgaatggaaa
gtgttgggtgtgacttccagctgatctggttctgtcaagtgtcgtggcacctcctgcacctgtgtggttcaggagagcagaaa
gtgggacacagagtggctgtgactctggggctggagtccacatcacggggagctgtgtctgtggctccagcatgatgccag
tgtcctgaggctgaatcacagctgcaggaagagccagctccagagaccatgtgtccagagtcctgcttctcactccatgtctat
gctgactggggtcatggatccattcggagtcttctcagtttagagagggagatagatacacaggctgcatacagagttctgg
acagagcacatttacactatttatacttgaagtgaaaatgcaccctaaagacagcagatgatggaccagttatgaagccccat
gaacaaggtgcaaacgctattgcagttccatgctcccgggttccagctgccaagcctcaattaactggcatttattatcatctaca
taaaactgagaaacacaaccagattgagcgaactgaatctgtagttggcattttgtcatttatttcaaaatggttgataaa
taataattgaacatagacatgagataaatgatattgttcaaaaatgactgtatcaaatgatggaattatgaagtattcttattgaca
tgctcatcacaataccacatgttccatggtcgaatgtcagtggtctcctgttttgaattcaatagaccaatattcatacgcgt

Figure 13b Unit 2 15699bp Seq ID 190

ggcgcgccgcaaccagttctagtcctggcggcgccggattctgtcccgggtgccctcttctaggccagctctctactgtgg
cccgggagtgcaaggatggcccaagagctgtgcctgcacccatgggagaccaggagaagcacctgactcctggct
tcggatcagcgtggtgcaccggttacagcggccattggagggtgaaccaacggtaaaggaagacatttctctctctctct
gtcactgtccactctgcttgcaaaaagtgtaccatttatataaaattgtctttatatttgcctcaaacacagagagactgaga
ctgacagtgatcttaattcctggcacttctcagatggctaccaagattcagctgggtcagcgcagccaggtgcttggaaat
ctgtccagggtcccacgtgggactacttggaaactcactatggcctcccagagggccttaggaaggaatagagactggaac
caactaggacacagcactcagcctcctacagagcaggaatccatcaggctcactccagtgtctcctgtcaccaccta
tcactcactgcaatgaaggaattgattcttccctgggattcagttgtttgagatctcaacattaggaaataggcttcaatcatatgat
aattaatctcaattatitaaagtatctatttttttagagacagtggtatagcaagagatgaagagaccaacagatggagagagaa
gcttctgtccagtggctcactctgtgaatggctgcaaaggctatgatggctgagcctaaagccaggagcctggaactcctccagt
ttcccacagggtggaaggggacaaacactttggctcatggctgacagtgatcaccattgcaatgcctacatctgtgagttcaattg
tggactgcatacaataatgtcacatactatgtgtccacatctacctggatcattcttttagtatgtatgtccaggctgacccatg
ttcctactgacagaacgacctccttctaatactgaataatattcctgtccaaatgtgaaagaatccattcactgttctgtgaacact
tgcttaattgtagactgaactattatgaacacagctgcattacctgaaccaggactgcgggtgaattgtgacacagtgactctac
ccagtgtgagctgaactcagagctcatgattacagaacagggaagagggggaacacagggcagctgataacaggacgcaa
agcaccactcacaggaaggaataattcgcctacaccacagtgaggctgactcagttcaccacaacccatgacaggttccacaaa
taactagaactgaggtctccaggcctcccacagagtgatggaaaggaggtggaatgtgatgacactgagttcatctgtgcc
cattctaccatgcacattccaatgacacaaaacccactaccatgtacagtgcttctgtgaggaacagtttaactaaagcaa
tggatgaataatgaacaacacatagttaaccttaaaaaatcaactgcagctcctcctctatttaaaagtgggcagcaggcattag
gacaaaattggctaaagtcccactagggtcaccctgttcccacagagaactgactgagcccagcctcacggctcccacact
ttcccataatgatcatgggaaactgtagatgacactacaggcaaaaggagcccctaccaccgacgttgaattacagacaca
gtttcttgcctcagggttcagaaagactaaccttaggggtcataggcatttgggatgtctagggtctcaacccaaaggcagaaa
tctcccattgtcacctttctgtccatctctacattctgtaactctcacccttctatctctctgtcccattgtcttcatatagatgat
aaggaaactaatgggatttaataaatgaggaacatgatattgtaacactggatattaagggtgcacacatataaaatgaacaa
ggtatctgccttcaactttttaaataattataacataaaaattcatatgatctgaatcatatcacagccatcaccgtacacccccaggt
caccacatctgcctggcgctgtcctgtctgagcgtctgacccatgcctgtatataggggcagctcatgcaaatggggcct
ccctgtgccatgaaaaccagcccagccctcaccctgcagctctggcacaggagctccagccccaggactcccagggtgtcca
ctcagtgatcgcactcaacacagacgctcaccatggagactgggctgcgctggcttctcctggtcgtgtgtcctaaaggtaatga
tggggaacgcgggacactgagcttgggagaggatgtgagtgagagacacagagatgtgagtgacagtgctctgacctgacatgtc
gtctgtgttgaggtgtccagtgtgaggtgcagctgggtggagctgggggagccttggtaaagcctggggggtcccttagactc
tctgtgcagcctctggattcacttcaagcctggatgagctgggtccgccaggctccaggggaaggggctggagtggggtt
ggcctgattaaaagcaaaactgatggtgggacaacagactacgctgcaccctgaaaggcagattcaccatctcaagagatgat
tcaaaaaacacgctgtatctgcaaatgaacagcctgaaaaccgaggacacagccgtgtattactgtaccacagacacagtgagg
ggcctcaggctgagcccagacacaaacctccctgcagggggcgcgcggctccaccagggggcgcacaaaacgcactgagc
ccagattctggctctgcacagggcagagtgagaccaagccctgggttctgtcagggttaggggttccctctcctgtaacagttt
tgagggaagaattctactttttgattctgtcctcccacttcaatgacggattgaaagttgatgtaaaggaagaagtcctaaatg
aacaattggatcatggacagcagctgtgtcaccaggatcagagatgtgggaatattgaggacactattgagctcttccaacct
gactcaggacagcaccctcagggcactgaaagccttggacagttcagtttccctgtataaccagggacagctgagacagggt
cgttgatctgttaacagcaactattccactcctcctcctgccacaaccagcaagtgtgactgaaccaaccttgactttatgt
gggtgatccacactcactttctgcagacttaaccgacctgcatttccctttaaactcctggaacagctgtggtccctgtgtgaact
cacagcactcaggaatgtactgcagctcagctgtgtctcaggctgggcttctgcaggcacctgggaaagcctacagggacttct
catccttaggtcctgggacctcctgtgggtccctgcctgaactcaggagggaaatgactggacatcagcagtcagggtca
tactgaatgaaagtgtgggtgtgacttccggctgatctgggtctgtccagtgatgaggaccctgcacctgtgtgtggtc
tgggagagcagaaagtgggacacacagaggctgtgactctggggctggagtcacatcacagggagctgtgtctgtgactc

Figure 13b Unit 2 15699bp Seq ID 190 (con't)

cagcatgatgccagtgctctgaggctgaatcacagctgcaggaagagccagtcaccagagacatgtatccagagtccttgc
 ctctctccatgtctatgctgactggcgctcatcaatgtatctcagctctttcatcagcttttagagagggagacaccaggtgcataca
 gagtcctggacagagcccatttacacaataccacactgtactgacaatgcaccctggaagacagtagatgatggatctgttatga
 ggccccatcaccaggtgggaacactgatgcagtccatgctccagggctccagctgccaagccttgagtgcactgccttcatt
 atcattctataaattccatataactctctttaaataactctgtccattgtcccattatccattccttctcaatgctaattcactgg
 attatgtgtggaactcctgagaatgacgctgctttaattgaacaggggactgcagagatggttgcacagtgactgcacatcccgt
 ggtctcctccctgatttcatattccttctaccaggcatggtgttgaggatcctgtgataattctatttctcagcttctgatgtttcca
 tgaagcttctcaatttacctgactccagccttaccaggttatcatctctcttctctgcaacaatgtgtcagctcctggtatgtctgg
 tgatactcagtctaaagaaagggatggggtatctcattgttctccaacatgtacgtgcatgatgccagatttaagcattttatac
 atttgctttatatatatttatttataaattacagatacaccttcacctctttaaacttgattgtgtattgtccaaaagggaaaagaga
 cagagactgacagacagtgatctactgaatcctggtaaactcccagatgccataaaaagctgagttgggtcagatcacaggtg
 catagaactcagtcaggctcaccacatgggataaactcagacttctcactgcctcccagagtgacattggcaggaatcctgaa
 attgggatctaaactgggacataaacctgagcatcctgatgcaggaggcaggaatcccacgtgatacctcaatgctgctcctat
 caccacctgtcagtcactgcatttaaaccattgattgctcccctgtgattcagttgcttgattcattcaacattggatctaggctc
 ctaatcatatgtacaattatcttcaattatttgaatatctatgtatgttgaagggcagtattatagcaagagaggagagacca
 cagagggagagagaagcttccatccactggtttctcctgaatggctgcaagggctgggcaagggcaagactgtagccagga
 gcctggaactctccagttcccacagagctcgaaggggacacacactgagtcatcgttgacagagttcctcctggtccatgagc
 agggagctggatttgaagtgcagcaagcaagatctgaaccatgctgctgagtgaaactgcatagggatggcagcttaacctgt
 ggggcccacaatgttggggagtaggcatgaggggtgctgcctgcattgaaggggaatacacccttagaaagaccttcaggcc
 ggccgcccggctcgcagactaatcctctactgcagtcccggcactccgggtctagtccaagttatggcggcgattctgctc
 tagttgctcctctccaagtcactctctgctgtgacctgggagtgacgtggaggatggccccagtgctgggccctgcacccctca
 tgggagaccaggaagaagcactggctcccggcttcgaataggcacagcgcgctggtggaggcggacattgggtgataaac
 caacggaaaggaagaccttctctctgtcttctctctctctctctctctgctgctcaaaaaaaaaaaaaagaaagaaagaaaa
 gaaagtaagaccttcaagtaacttaattttaaatttgattataacacatgaatcagagaggagaatgagagaaagagagatgtc
 cgacctgtgcttcccuaatgctgctggccaggctgtcctccagcttagcccggctcaggaaaacaatcaggctgcacacac
 aagcgtgacagggccccaaaattcctcccataaactctgggtcctggggctgcaggagcaggaaactggtactgggagccaga
 gccacacatgggacctgcactctctgggggatgtgctcatcttaaacagtgcuaattcaatttattttcttatttccatgaa
 atttgatgactcagatgcacctacgtgaaacctggctcctgtgcatctctctgttaaaaaatggaatccagcaaaaagtgctccttgt
 ctgctgctggcaggaggtctgcagacctaccaggaagccccagtgagtgcataagggtggactggggcagagctgagtcct
 gtggtgaggggcatggatcaaggctgtgggagctcctctaccacactggagtggggacacatccacactgtactgatgattct
 cctgtaacgttgaatggaccaagacctaaaggaacctatgaatacatttaggtccattcacataaaaggaccaagagagg
 gttcagtgatgaatgagagcatacaatttactcactcaccacatgaataatgtcagactgccccgggacactctcatcagctc
 gactatcatactaaaggagtctgacgccagggtcctatatagggggcagcatgcaaatagggtcctctgcccagaaaa
 ccagccagccctcactctgcagctctggcacaggagctccagccccaggtcctcactcagtgatgcactcaa
 cacagacgctcaccatggagactgggctgctgctgcttctctgctgctgctcaaggaatgaagggccagtgcaggag
 ctactaggctaattcctccgctttcggcgccagcacacagggcttagtcccgggtggggcctggattctgtcccgggtgccc
 ctctccaggccagctctctactgtggccagggagtgaagtggaggatggccccagtaacttgggcccctgcacccatgggaga
 ccaggataagctgctcctgccatcggatcatcgcggtgagccggcctcagtgagctgacacggtgaccattggaggggtg
 aagcaacggcaaggaagaccttctctctctctctctctcactgtccactctgctgctcatataaaaaaggaatgatggagaa
 tgcaaggcactgagtttgggagaggatgtgagagagagtgtagtgacagttctgtgatcgtctgctgcttgcaggtgtcca
 gtgtgaggtgcagctggtggagtctgggggaggttggtccagcctgggggggtccctgagactctctgtgcagcctctggatt
 caccttagtagctattgatgagctgggtccgccaggctccaggaaggggctggagtgggtggccaacataaagcaagatg
 gaagtgaataactatgagactctggaaggccgattcaccatctccagagacaacgccaaagaactcactgtatctgcaaat
 gaacagcctgagagccgaggacacggctgtgtattactgtgcgagagacacagtgaggggcccctcaggctgagcccagaca

Figure 13b Unit 2 15699bp Seq ID 190 (con't)

caaacctccctgcaggggcgcgcggctccaccagggggcgcacaaaacgcactgagcccagattctggctctgcacaggcg
cagagtgagaccaagccctggttctctgacaggatttagggttcctctcctgtaacagtttgagggaagaattctacttttgatt
ctgtccctccaactcaatgacggattgaaagttgatgaaaaggaagaagtcctaaatgaacaattggatcatggacagcga
ctgtgctcaccaggatcagagatgtgggaatattgaggacactattgagctcttccaacctgactcaggacagcacccctcagg
cactgaaagcttggacagttcagtttccctgtataaccaggacagctgagacagggtcgtgatctgtaacagcaactattc
cactcatccctctctgccacaaccagcaagtgtgactgaaccaaccttgactttacatgtgggtgatccacactcactttctgca
gactttaaccccgacctgcatttcccttaactcctgaaacagctctgtggctcctgtgtgaatgtcacagcactcaggactgtactgca
gctcagctgtgctcaggctgagcttctgcaggcacctgggaagcctacagggacttctcctccttaggtcctgggacctcct
gggggctccctgcatgaactcaggagggaaatgcgatggacatcagcggtaaggctcactgaatggaaagtgtgggtgt
gcactccagctgatctggttctgcaagtgtcgtgggaacctgcacctgctgtggtcaggagagcagaaagtgggacac
acagaggctgtgactctggggctggagttccatcacggggagctgtgctgtggctccagcatgatgccagtgctctgagg
ctgaatcacagctgcaggaagagagagtcctcagagaccatgtgtccagagtcctgctctcactccatgtttgtgctgactggg
gtcatcaattattggagctttcattaatattacaggctgcatatcagagtcctggacggagcccatttacacattcaccacactgt
actgacaatgcacctggaagacagtagatgatggatctgttatgaggccccatccaagggtggaacactgatgaagtccat
gctcctgggttcagctgccaaagccttgagtacaatggcctttattaccatctccataaaacattggcaagcacaccagtatttcc
gttttgcgttctttataaatgtttataaataaattggatatagacatatgaggtgcaaggatattgttcaaaattgaatgtag
caaattgatggaattatgaagtcattctattgacatgctcacaacaaatccacatgttccatggtagattgacagttgcttctgttt
ggaattctgtagacacaatattgcttaactgctgacacctgctgtacagtagatccctcaacatgtggatcctgtgggactgaaac
tctgacctgctccaatgtctcctctgttccatgccctttcttccattattgattccttctccatgaaaattcactggattatgtgtga
acactcgtgaacgacgctgcttaattgcacatggaactgcagttgtagttctatagagactgtgaatccatgcacatctccctgg
gattcacallctgctacccaaaatgctgtttgaagatcctgtgataactgtatgtctcagttttgatgactttccataatggattact
aattacctgactccagcctgtacaaccagagtgteatctctcttccacaagaattgtcagctctggcttctctgtaatactca
ttcaacatgaaggatggacatctcattgtcttcaaaatgtgcacatgatgatctcagattatggcattttacatgtttgctttat
atcatttctaaatttatagatgtaactacagttaaaaatcatagattttatftaaaattgtctttatgtattatctaaaaccacaaag
agactgagactgacagtgagattctatctctggctactaccaagagggtcacaacatgcttcagttagatcaggtgacagccagg
ggcttggaaattctgacaggtctccacatgggagcttggaaactcactatggccacgagaaccaaactaggacacagcactaa
agaacctgattcggggccggcgcggctcactaggctaatcctccgcttccggcaccggcaccgggttctagctccg
gtcggggcggcgtattctgtcccgggtgcccctctcaggccagctctctgctgtgcccaggagtgacagtgaggatggccc
agggtgctggccctgcacccatgggagaccaggagaagcactggctcctgccatcggatcagcgcgggtgcccagccg
cagtgcgacggccgcggcggccattggagggtgaaccaacggcaaaaggaagaccttgtctctgtctctctcactgtcc
actctgctgtcaaaaaaaaaaaaaaaaaaagaacctgacacaggaggcagcaatccacatgtaactccagtgtgctcctgt
caccacctatcagtcactgcatttaacaattgatttctccctgggattcacttctctgtgtttattcaacattgcatataggctccta
atgatalggataaataatcttctatttttaataatctattttttg.ittgaaaggcattgttatagcaagagatgtcgtctgtgtttgcag
gtgcagctgggtggagtctgggggaggcttggcaagcctggagggtccctgagactctcctgtgcagcctctggattcacctca
gtgactactacatgagctggatccgccaggctccagggaaggggctggagtggtttcatacattagtagtagtgtagtaccat
atactacgcagactctgtgaaggccgattcaccatctccagggacaacgccaagaactcactgtatctgcaaatgaacagcct
gagagccgaggacacggccgtgtattactgtcgagagacacagtgaggggcccctcaggctgagcccagacacaaacctcc
ctgcagggggcgcacaagacacactgagtgacagattctggctatgcataagtgcagaaggagcccaagcctggttctctgct
agggaactggggttccctctctgcaacagttttgagggaagaattcttctttgatgattctgacctgtactgactctctgatgtgac
aggagaagctctacatgaacgaatttaggtcagtggtctccagtatcagagatgtgggacaatctggggacttagtgagtggtg
ccagcctgagtcaggacagagttcataggacactgccacttagggcaattttacttttcccttcaatccagcaatagctgagcc
agggttgagtttaaaatctgctatttaagtatctctctcttcaaacgagcactgtgctgaactgaaccaaccgtctattttaa
actgtgagtggtctgcatcatttcaacagactttatcccgatctccattttccttcaatccagcaatagctgagcc
gtcacagcactcaggaatatactgcagctcagttgtgtctcaggctgagcttctgcaggcacctgggaagcctacagggacttc

Figure 13b Unit 2 15699bp Seq ID 190 (con't)

ctcattcttaggtcctgggacctcctgtgggctccctgcctggactcaggaggaaaaacactggacatcagcagtcagggt
catcactgaatggaaagtgttggcgtgcacttccatctggcttagtccgctccagtgctgtgagaccacctgcacatgtgtggtt
caggagaggagaaagtgggacacacagtggctgtgcactctggggctggagtccacatcacggggagctgtgtctgtggct
ccagcatgatgccagtgctctgaggctgaatcacagctgcaggaagagccagtcacagatcatgtgtccagagtcctgct
cttcaattcatgtctatgtccaaatgtcaatccattggagctttcattaacattacagtgaggagtcactcaggctgcatacatt
gctggatggagcccatttcacgcttccatactgtactgacaatgcaccctggaagacagtagatgatggatctgttatgagcc
cccataccaaggagggaacactgatgcagttccaagctcctgggtccagctgccaagcctgagtgactgccttttaaatcat
ctccataaaacattggcaaacacaataccagtagcagagatctgaatctcatgactggccattgttcatcctttatcccatgtagc
tacctacatcacatattatgtgtctcctggcctggatggttagcagagtgacttttagtgacatattatcctaggcacattatctcca
gggtcatccaagtgtccaaaattacagaatttcttactctgtactgctttaaactcctttaaaatgtaacttgtccatttctcatttc
cattctttctccatgagatgtcactggatcatatgtgtgaactctttgaatgaactctttaaatgaacaaggaactgcagaggtgtt
ggcacagtgcagatcccgtggacacctccctgggattcatattcattgtaccatgcaatggtgcttgaagatcatgtaataa
ttctgttctagttttagtctttccatacggtttactaattaacctgacaagacttctaccagggttatcatctctctaccctcataa
aacatgtcagctctggtatgtctgataatagccattctaactggagcagatgcagatgtctcattgtgctccaacatggacatacatgt
ggtcccagattatagcattttacatgtttgcctatatatcttctttaaataattatagatattatataaattttaaattatcattttattga
gaactgtctttatgtattgtacaaaaccacagagagactgagactgtcagtgatcttctaattcctgcgtcacttcccactggcta
caaaagatttctgctgggtcaggtgacagccaggtgcttggaaattctgtccagggctcccacgtgggagacttggaaactacact
acgctcccagagtgctcagggcaggaatgacactggaaccaacaaggacacagcactaagcatcctgatacgggagg
caggaatccctcaggctcactccagtgctgctcctgtctccacctatcatcactgcagatgacacaattgatttctccttgggattc
agtgctgagatcattcaacattaggaacaggctcctaatcatatgtacaattaatcttcaatgtattttaaagtatctattttatttt
acaaacagtgtatagcaagagaggaagagatcaacaaatggagagagaattccatccactggtaactcctgaatggctgca
aaggctggggatggctcagcctaaattcaggagcctgacttggatcatggtagacagtttaccattgcactgcctacctgtg
agttcaagcttgtgcactgcagatgataactaatgtcacatactgtgtgccacatctacctggatcatttcttttagtatgaatgctccag
gctgaccacatttctaatgacagaacaacctccttctaatactgaataatattcttagtctaatgtgaaagaatccattcactgttt
tatgcacacttgtctttgtagactgaactattgtgaacaaagctgcattacccaacgcaggactgcgggtgaattgtacacagt
gacttctaccagtgctttagctaaactcaaaagctatggttacagaacagggaaagcgtggccggcgtgtggctcactaggcta
atcctccgcttgcggcgccggcacactgggttctagtcacgggtgggcaacggattctgtcctgggtgccatcttccaagcc
agctcactgctgtggccagggagtgactggaggatggccaagtgttgggacctgcacccatgggagaccaggagaagt
acctggctcctgccatcggatcagcgtcgtgcgccggccacagcacgccaatgcaacggccattggagggtgaaacaacgg
caaaggaagaccttctctctctctctctctcttactgttactctgcctgtcaaaaaataaaaaaagaacagggaagaggaa
cacagggcagctgatacaggggcagaaagcaccacaaaggcaggaataattctgccacaccttgggctgactcagttc
accacaacacacaacaggtccaagaatgactagaactgaggtctccaggcctcccaacagagtgatggaaaggaggtggaa
atgtgaatgacagtgagttcatctgtgccatttctaccatgcacattccagtagcacacaacccccactaccatgtacagtgctctg
tgttcaggaacagttttaaacagtttaaacagttttaaactttaaaggcaatggatgaataaggaaaaaacatagttaaccttaaaaa
attcacttgagtattcatctattttaaagtgggtgacaggcattaggacaaaattggctaagtccccactagggtcacctgtgtcc
catcagagaactgactgagcccagcctcacggcttccacactttccctcataatgcatcatgggaaactgtagatgatgctaca
agcaaatgaaccctaccaccgacgttgaattacagacacagtttattgtccaggttcagaaagactaactcctagatgtcat
aggcatttgggtgtctggggctgtcaacccaaaggcataaattcccctgtcactttctgtccatctctacatttctgtctctca
ccccctctctctatcactgtaccaatgtctttcatatagatgataaagaactaatggttatttaaataaatgaggacatgatattgt
aacactc gatatgaagggtacacacataftaaatgaaacaaggtcattgccttcaagttttaaataatgataacataaaaattcac
atgatcttaattctataaccatcccaacacaccaagaacacagacaactcccagggccctgtcctgttgaggcatctgac
cccagcctgctatctaggggcagcacatgcaaatagggtcctctgcccataaaaacctgcccagcctcaccctgcagctc
tggcacaggagctccagcctcaggactcccaggtgtccacacagtgacacagacgctcaccatggagactgg
gctgcgctggcttctcctggctgctgtgctcaaggtaatgatggagaacgcgggacactgagcttgggagaggacatgagtg

Figure 13b Unit 2 15699bp Seq ID 190 (con't)

agagacacagagagtgtgcgtgacagtgtcctgatcatgtcgtctgtgtttgcaggtgtccaatgtgaggtgcagctggtggagt
ctgggggaggcttggtagaccagggcggccctgagactctctgtacagcttctggattcaccttggtgattatgctatgagct
ggtccgccaggctccaggaaggggctggagtgggtaggttcattagaagcaaagcttatggtgggacaacagaatacacc
gcgtctgtgaaaggcagattcaccatctcaagagatggttccaaaagcatcgcctatctgcaaatgaacagcctgaaaaccgag
gacacagccgtgtattactgtactagagacacagtgaggggcccctcaggctgagcccagacacaaacctcctgctaccgttcg
tatagcatacattatacgaagttatggccggccacgcgt

Figure 13c Unit 3 13971bp Seq ID 191

ggcgcgccggaagcctacagggaactcctcactccttaggtcctgtttctctcctgtggggctccctgcctgaactcaggaggga
aatgcaccggacatcagcgggtcaaggatcactgaatggaaagtgttggtgtgcactccagctgatctggtctgctccagtg
tcgtgggaacccctgcacctgctgtggtcaggagagcagaaagtgggacacacagtggtctgcactctggagctggagtc
cacatcacggggagctgtgctgtggtccagcatgatgccagtgtcctgaggctgaatcacagctgcaggaagagccagtc
ccagagacctatgtccagagtcctgctctttactccacgtctatgctccaaaggtaatccattggagctttcattaacattca
gtgggagtcactcaggctgcatacagagtgtggacagagcccacttacaggctttccatactgtactgaaatgcacctgga
agacagtagatgatggatctgttatgaggccccatccaagaagggaacactgatgcagttccatgatcctgggtccagctgc
caaacctgagtacactgcctttattatcactccataaaacattggcaaacacaacaccagattcagagatctgaatctcatgac
tggccattgttcatcctttatccatgtaggtagcatcacatattatgtcttctggcctggatgcttagcagagtgtactttactg
acatattatcctaggcacattatctccgggatcatccaagtggtccaaaattacagaattcttactctgtactgcttgaaccttctt
taaatgtaacctgtccatttcttccatttcttccatttcttccatttcttccatttcttccatttcttccatttcttccatttctt
ctttaaatagaacaaggaaactgcagaggtgttgccacagtgactgtagattccgtggacacctccctgggattcatattcattgtac
catgcaatggtgctgaagatcatgaataattctgttctagttttgatgctttccataatggtttactaattaacctgacacagactt
gtaccagggtatcactctctctacctccataaaacatgcagctctggatgtctgataatagccattcctaaggaaggatgcgat
gtctcgttgcttcaacatggacatacatgatggcccagattgtagcatttatatgtttgtctttatcttctttaaattataggt
gtaccataaaattttaaattatcattttatataaaattgtctttatgtattgtccaaaaccacaaagagactgagactgacagtgatc
tttaactcctgggtcacttccccaatggctacaaaagcttcagctgggtcaggtgacagccaggtgcttgaattctgtccaggg
ctcccacgtgggactacttgaacttcacctacggcctcccagaggggcttaggaaggaaatgacactggaaccaaactagg
acacagcactcagatcctcatacaggagcaggaatccatcaggctcactccagtgctgctcctgtcaccacctatcactcactgc
atttaaggaattgatttctcccttgggattcagttactgagatcttgaacattaggaataggctcctaatacatatgataatctcaa
ttattttaagatctatttttttagagacagtggtatagcaagagatgaagagaccaacggatggagagagaagcttctgtccagt
gggtcactttgtgaatggctgcaaggctatgatggctgagcaagaaaccaggagcctggaacttctctagtttcccacagggc
tgaaggggacaaacacttgggtcatggttagacagtgatcaccattgcaatgcctacctctgtgagtcaattgtggactgcatat
acaaataatgtcacatactatgtgtccacatctacctggattatcttttagtatgtatactccaagctgacctatgttctactgaca
gaacgacctccttcttaacactgaataataatcctatgtccaagtgttaaagaatccattcactgtttatgctgacttgttctttagact
gaactattgggaacacagctgcattacctgaaccaggactacgggtgaattgtgacacagtgacttctaccagtgctgagct
gaactcagagccatgattacagaacagggaagagggggaacacagggcagctgataacaggacacaaagcaccactcaca
ggaaggaaataattctgcccacaccacagtggtgactcagttcaccacaacacacgacaggtccacaataactagaactga
ggctcaccaggcctcccacacagatgatgaaaggaggtgaaatgtgcatgacactgagttcatctgtgcccatttctaccatgc
acattccaggagcacataaaccctaccatgtacagtggtctgttcaaggaaactttaaactaaggcaatggatgaataaat
gaacaacacatagtaaccttaaaaaatccacttcaacttctctatcttataaaagtgggtgacaggcactaggacaaaattggctaa
gtcccactagggacaccgtgttcccacagagaactgactgagcctagcctcacggctcccacactttccctcataatgcaa
catgggaacctgtagatgacgtacaagcaaggagcccctaccaccaacggttgaattacagacacagttcttctcagggt
tcagaaagactaactcctagggtcataggcattgggatgtatggggctgtcaacccaaggcagaaatctcccattgtcaca
ttctgtccaactctacattctgtaactctacccctctctctctctctgtccaatgtcttcatatagatgataaggaaactaaagg
ttatttaataaatgaggaacatgatattgtaacactggatattaagggtacacattgaattaagatgaaataatgtattgtcttcaa
cttttaataaatgataacataaaaatcactgatctgaatttatcacagccatcagcgtacacccccaggcaccacatctgcc
ctgggcccctgtctgtctgaggcatctgacccatgctgttattagcagcagcagatgcaaatggggcctccctctgcccataga
aaaccagcccagccctcacctgcagctctggcacaggagctccagccccaggactcccagggtgctcactcagtgatgcact
caacacagacgctcaccatggagactgggtcgcgtggcttctcctggctgctgtgctcaaaggtaatgatggagaacgcggg
gcactgagctgggagaggatgtgagtgagagacacagagagtgagtgacagtgcttgaccatgtcgtctgtgttgcaggt
gtccagtgtaggtgcagctgggtggagctgggggaggctgtgctcagcctggggggctccctgagactctctgtgcagcctct
ggattcacctcagtagctatgctatgcactgggtccgccaggtccagggaaggagactggaatatgtttcagctattagtagtaat

Figure 13c Unit 3 13971bp Seq ID 191 (con't)

ggggtagcacatattatgcaaactctgtgaaggcagattcaccatctccagagacaattccaagaacacgctgtatcttcaa
gggcagcctgagagctgaggacatggctgtgtattactgtgcgagagacacagtgaggggcccagcctgagcccagacac
aaacctccctgcagggatgcgcggctccagcagggggcgcctcaagacgcactgattgcagattctgatctgcacaggcgcag
aaggagatgaagccctggtttctgtcagggattggggtttccctctcctgtaacagtttgagggaagaattctactttttattctgt
ccctcaaactcaatgacggggagaaaagttgatgtaaaaggagaagtcctaaatgaacaattggttcatggacagcactgt
gctcaccaaagcagagatgtgggaatattgaggactctattgagtcttccaacctgactcaggacagcaccctcagggcact
gaaagcttggacagttcagtttccctgtataaccagggacagccgagacagggccgtgatctgtaaacagcaactattcca
ctcaccctctcctgccacaaccagcaagtgtcactgaaccaactttgactctacatgtgtgatccacactcactttctgcaga
ctttaaccgacctgcatttcccttaacctggaacagctctgtgtccctgtgtgaacgtcacaacactcaggaatgtactgctgt
cagctgtgtctcaggctgagcttctcaggcactgggaagcctacagggactctcctcctctaggtcctgggaccctcctgt
gggctccctgcctgaactcaggagggaatgcactggacatcagcagtcaggctcactgaatggaaagtgtgggtgtgca
ctccggctgatctggttctgctccagtgtcagggaccccctgcacctgctgtggttcaggagagcagaaagtgggacacac
agtggctgtgactctggggctggagtccacatcacggggagctgtgtctgtggtccagcatgatcccagtgtcctgaggct
gaatcacagctgcaggaagagccagtcccagagaccatglatccagagtcctgctctctctccatgtctatgctgactggcgtc
atcaatgtatttcagctttcctcagtttagagagggagacaccaggctgcatacagagtcctggacagagcccattacacat
ttctcacttctgactgacagtgacctggagacagtagatgatggatctgtatgaggccccatccaagggtgggaacactg
atgcagttccatgctcctgggtccagctgccaagccttgagtacactgcctttattatcctccataaaacattggcaaacaca
caccagttcagagatcttaacttcatgttttgcctcagttcaccacaacctatgacatgtccaccaataactagaactgaggtt
ccaggcctccaacagagtgatggaaaggaggtggaaatgtaatgacactgagttgatctgtaccatttctacaacacacag
attccaatagcacactacaccccactaccatgtacagtggtccgtgttcaggaacagtttaactaaagcagtgaaatgaataagg
gaacaaatcatagttaaccttaacaaatccacttatagctttcctctgtttaaaaagtggtggcaggcattaggacaaaattggct
aagtcctccgctagggacaccatgttcccacagattgttgcctgagccatectcacagttccaacttttctgataatacatc
ttgggaaactgtagatgacgctacaagtgaatgagaccctaccaccgatgtggacacacagtttctgctccaggtttagaag
tctaactcctagatgtctcaacattcatgtttctcactcctctctctctctgctgctgtttcaatgtcattagaatagttaataatgaacca
atggttatttataaaatgaaggacatgatactgctaactggatataaagagtacaaacattaaagtgaatgaggtattgccttca
aatgttataaaataataacataaaatttgccttgatctgaatcctatcatagccatcaccacacaccaccaggacaccacatctgc
cctaggcacagtcctgtctgaggcatctgacccatgcctgctataggggcagcagatgcaaataggcctccctctaccat
gaaaccagcccagccctcaccctgcagctctggcacaggagctccagcccaggactcccagggtgctcactcagtgattgca
ctcaacacagacgctcaccctcagagactgggctgtgctagcttctcctggctgctgtgctcaaaggtaatgatggggaacgc
ggggcactgagctgggagaggatgtaagtgagagacacagagagtgactgacagtgctcctgacctgctgtgtgtttgc
aggtgtccagtgtaagtgcagctggtggagctgggggagctgtgttacagcctggggggtccctgagactctcctgtgcag
cctctggattcactttgatgattataccatgcactgggtccgcaagctccggggaagggtctggagtgggtctctcttattagt
ggatggtgtagcacatactatgcagactctgtgaaggccgattcaccatctccagagacaacagcaaaaactccctgtatctg
caaatgaacagctctgagaactgaggacaccgcttattactgtgcaaaagatgacacagtgaggggcccctcaggctgagcc
cagacacaaacctcctgcaggggagtgaggctccaccagaggcgcgcaagacgcactgagcagagattctggctcctgctc
aggcgcagaggagatgaagccctggttctgtcagggattgggttccctctcctacagtttgagggaagaattctcctttatg
actctgtcccttcttgaactcactgacgtagagaagttgaagtgacaggaagaagtcctaaacggacacattcaggtttgtgaa
cgatgacagtggtcaccaggatcagagatgcaggactatctggggacctgtgagtggtccagctctgactcaggacagcgtt
ccaggacactgccgaclataagacaatttggtttccctcaaacccagacagaccgagccagagctctgattgctaccicagttt
tataagtcgtcccacctcctgtcagagcagcctgtgtgctgaactgaaccaaccgtcttattttaaactgtgggtgtctgcattcac
ttctacagactttatacccaatctccatttcccttaacctggaacagctgtggtccctgtgtgacttccagctgatctggttctgc
tccagtgctatgggaccccctgcacctgctgtggttcaggagagcagaaagtgggacacacagtggtctgtcactctggggc
tggagtccacatcacggggagctgtgtctgtggtccagcatgatcccagtgtcctgaggctgaatcacagctgcaggaaga
gccagtcacaagagaccatgtgaccagagtcctactctctccatgtctatgctgactggcgtcatcaatgtatttctcagtttca

Figure 13c Unit 3 13971bp Seq ID 191 (con't)

tcagtttagagaggagacaccaggctgcatatcagagtcctggacagagcccatttacacaataccacacttgaactgaca
atgcacctggaagacagtagatgatggatctgttatgaggccccatcaccaaggtgggaacattgatgcagttccatgctctg
ggtccagctgccaagccttgagtacactgccttcatttateatctccataaacattggcaaacacaacaccagtattcagagatca
atcgtcatgattggccatcattgctatttcatattcctaaatgttgataaataatattgagtatagctctatgaagtacaatgatatgtg
ggtcaaaatgaatgcatactgagaggaattgtgaagtcagacttgatgacacattcataccacaacacatgttttggaggattca
cctgctctctgcttaagagatttctactcacaatattcttaacctctgaccatgctgtgcagtagatccctcagtggtggagc
ctgtgggactgaaactctgacctgctccagcgtctcctctgttctccctcgtcagactttggtcttaccattgtactttctgctct
gtgagttcagcctttatcccatgtaggtagcatcacatattatgtgtcttctggcctggatgcttagcagagtgtactttactgaca
tattatcctaggcacattattctccgggaccatccaagtggtccaaaatcacagaatttctactttactgctataaacttcttttaa
aatataactttgcccatttcttcataatccattctgttccatgagatgctactggatcatatgtgtgaactctttgaatgacggtgctt
taaatgaacaaggaactgcagaggtgtttgccacagtactgtagatcccgtggacacctccctgggatttcatattcattgtacca
tgcaatggtgctgaagatcatgtaataattctgtttctagttttgatgctttccataatggtttactaattaacctgacacagactgt
accagggtatcctctctctacctccataaaacatgctagctctggtatgtctgataataggcatttaacaggagggtgggat
gtctcattgtgcttcaacatggacatacatgatggtcctagattgtagcattttatattgttctttatattcttttgaatttatagat
gtaacataaaatttaaaatattatcattttattggccagcgcattggctcactaggctaactctccacctgcccgaactgcaactccgggt
ctagctcggcgggctggttctgctctggttggccctctccagctcctctactgtggcccaggagcgcagtgagg
atgatccaagtgtggccctgcaccgctggagaccaggagaagcacctggctcctggctacggatcagcgcggtgacg
cggctacagcggccattggagggtgaaccaacgtaaggaagacattgctctctgctctctcactgccactgtccactctgcc
catcaaaaaatattatcattttatttaaaaatgtctgtatgtattgtccaaaaccacagagagactgagactgacagtgatctcta
actcctgggttacttctgatggatacaaaatcagctaggctcaagcgcacatccagttgcttggaaattctgtccagggtccacgtg
ggactacttgaactcactacggcctcccagaggggcttaggcaggcatctgaaaccggaaccatactaggacacagcact
aagcatcctgacacgggagggcaggaatccctcaggctcactccagtgtctcctgtctccacctatcactgcatttaagga
attgattcagttgctgagatcttcaacattgggaaataggctcctaatacatatgtataatcaatcttcaattactttaagtatctattat
ttattttaagacagtgtatagcaagagaggaagagaccaacagatggagggagaattccatccactggtaacttctgaatgg
ctgcaaaggctagggtgagcctaaaagtcagacttggaaactccaccagtttcccacagggaggaaggggacaacc
cctggtcatggtagacagtatcaccattgctcttctctctgtgagttcaactgtggagttcaactgtgcatgtataactaatgt
cacatactatgtgtccacatctacctggattattctgttagcatattatgtccaggctgaccacgttccctaatgacagaacaacc
tcttcttaatactgaataatattctatgtccaaatgtgaaataatccattcactgttfaatgcacacttgatttattgtagactgaactatt
atgaacaaaactgcattacctgaaccaggactgcgggtgaattgtgacacagtacttctaccagtgttgagctgaactcag
agccatgattacagaatagagaagacagtggaacacagggtggctgataccagggcacaaaacaccacaaaggcaggaat
aattctgcccacaccacagtgggctgactcagttcaccacaaccacagaggtccacaaataactagaactgaggcctccag
acctcaaacacagagtgatggaaaggaggtggaaatgtgctgacactgagttcatctgtgccatttctgcatgcacattccaa
tagcacacaaacccactacctgtacagtgttctgtgttcaggaacagtttaaacagtttaaacagtttttaatttaaggcaatg
atgaataaggaaacaaaacatagttatcctaaagatatccattgagtccttcatctgtttaaagtgggcgacagccattaggac
aaaattggctaaagtcaccactagggtcaccgtgttccatcagagaactgactaagcccagcctcaggcttcccacactttcc
ctcataatgcatcatgggaaactgcaggtgacactaagagcaaatgaggccctaccactgacattggaattacagtcacatttctt
gtccagggtcagaaagactaactcctagggtcataggcatttgggatgtctgggctgtcaacccaaaggcagaaatctcc
ctttgtcacttttctgtccatctctacatttctgtatctctcacccttctctctatcactgtcccaatgtcttcatatagatgataagg
aactaatggttatttaataaatgaaggacatggtattgtaatactcgatatgaagggcacacacatattaaaatgaacaaagat
ttcctttaacttttaaaataatgataacataaaaatcattatgatctgaattatcacagccatcaccgtacacccccagggtacca
catctgccctgggccctgtcctgtctgaggcgtctgacccatgcctgtatataggggcagccatgcaaatagggtccctc
tgccatgaaaaccagcccagcctcaccctgcagctctggcacaggagctccagcccaggactcccagggtgtccactcag
gatgcactcaacacagacactccatggagactgggctgcgctggcttctcctggctgctgtgctcaaggtaatgatggag
aacgcggggactgagctgggagaggatgtgagtgagagacacagagagtgtagtgacagtgctcctgacctgctctgt

Figure 13c Unit 3 13971bp Seq ID 191 (con't)

gtttgcaggtgtccagtgtgaggtgcagctggtggagtctgggggaggcttggtacagcctgggggggtccctgagactctctg
tgcagcctctggattcaccttcagtagctatagcatgaactgggtccgccaggctccagggaaggggctggagtgggtttcatac
attagtagtagtagtagtaccatatactacgcagactctgtgaagggccgattcaccatctccagagacaatgccaagaactcact
gtatctgcaaatgaacagcctgagagccgaggacacggctgtgtattactgtgcgagagacacagtgagggggccctcaggctg
agcccagacacaaacctccctgcaggggagtgccggctccaccagggggcgcgcaagacacactgagtcagattctggtcct
gctcagggcacagagggagatgaagccctggttctctcagggatttgggttctctctacagttttgaggggaagacttctactt
caggattctgtcccttactgacgccactgacgtagagaagctttatgtgacaggaagaagtcttaaatggacacattcaggtttg
tgaacgatgacagtggcaccagggtcagagatgtgagacaatcaggggacctcgtgagtgttccagtctgactcaggaca
gcgttccaggacactgccgactataagacaatttagtttcttcaaaccagacagaccgagccagagctttgatttctacct
cagttttataagtcgtccatctctctgtcagagcagcctgtgtgctgactgaaccaaccgtcttattttaactgtgggtgttctgca
ttcactttctacagactttatacccaatctccatttcccttaatccctgaaacagctctgtgtccctgtgtgacttccagctgatctggt
ctgctccagtgtcgtgggaaccctgcacctgctgtggttcaggagagcagaaaagtgggacacagagcggctgtgactctg
gggctggagtccacatcacggggagctgtgtctgtggtccagcatgatgccagtgctctgaggctgaatcacagctgcagg
aagagccagctccagagaccatgtgtccagagtcctgctcttactccatgtctatgctgactggggatcaatccatttgtgtt
ttcatcagtagtagagagggagacaccaggtgcatafcacattgctggatggagcccatttgcacgctttccatacttgtact
gacagtgcacctggaagacagtacatgatggatctgtatgaggccccatcaccaaggtgggaacactgatgcagtttcatgct
cctgggttccagctgacaagctgagtcactgcctttattatcatctccataaaacatggccaacacaacaccagtttcaaatg
caatcgtcatgattggccatcgttctatttataatcctaaatgtttgataaataatattgagtagctctatgaggtacaatgatatg
gtggacaaaatgaatgcatattgagtggaaattatgaagtcaggcttgatgacacattcataccacaacacatgttttggaggatc
acctgtctctgctcttaagagatttccactcacaatattctcttaacctctgtcaccatgctgtgcagtagatccctcagtgtgtgga
gcctgtgggactaaaactctgacctgtccagcgtctctctgttctccctcgtcagactttggtcttaccattgtacttctgc
ctctgtgccttccagcctttatcccatgtaggtagcatcacatattatgtgtcttctggcctggatgcttagcagagtgtactttactg
acatattatcttaggcacattattctccgggatcatccaagttgtccaaaatcacagaatttctactctgtactgcttttaacttcttt
aaaatgtaactttgtccatttcttccatttcttcttccatgagatgtcactggatcatatgtgtgaactctttgaaatggcgggtgc
tttaaatgaacaaggaaactgcagaggtgttggcacagtgactgcagatcccgtggacacctccctgggatttcatattcattgtac
catgcaatggtgctgaagatcatgtaataattctgttctagttttgatgctttccataatggtttactaattaacctgacacagact
gtaccaggttatcatctctcttacctccataaaacatgtcagctctggtatgtctgataatagtcattctaacaggaggatggga
tgtctcattgtgcttcaacatggacatacatgatggtcctagattttagcattttatgtttgtctttatcttctttgaaattatagat
gtaacataaattttaaaatttatttattggccagcgcagtggtcactaggctaatctccacctgcgggtgacttaccgttctgata
gcatacattatacgaagttatggccggccacgcgt

Figure 13d Unit 4 15445bp Seq ID 192

gcgcgccgcgatcgcgaagttcctattccgaagttcctattctctagaaaglataggaacttcagggcgcgaggctctttaggg
ggcgcaacaagacgcactgagcccagattcctgtcctgcacagggcgcagagggatgaagccctggttccctacaggaatt
ggggtttgctgtcctgtaacagtttgaggaagaattctacttttgattctgtccctcccaacttcaatgacggagagaaagttga
tgtaaaaagaagaagtcctaattgaacaatttggtcatggacagcactgtgtcaccagggtcagagatgtgggaatattgag
gacactattgagtcttccaacctgactcccagacagcccccagggcactgaaagcttggacagttcagtttccctgtataac
caggacagccgagacagggcgttgatctgttaaacagcaactattccactatccctctctgccacaaccagcaagtgtgca
ctgaaccaactttgactctacatgtgtgcgatccacactactttctgcagacttaacccgacctgcatttcccttaactcctggaac
agtctgtggctcctgtgtgaacgtcacagcactcaggaatgtactgcagctcagctgtgtctcgggctgagcttctgcaggcacc
tgggaagcctacagggacttctcctcctctaggtcctgggaccctcctgtgggctcctgctgaactcaagagagaaatgtgc
tgacatcagcagtcaggatcataactgaatggaaagtgtggatgtgcacttccagctgatctggttctgctccagtgccatggg
acccctgcacctgctgtggtcagggagagcagaaagtgggacacacagtggctgtgcactctggagctggagtccacatca
cggggagctgtgtctgtggctccagcatgatgaccaatgtcctgaggtgaatcacagctgcaggaagagccagtcttagacca
tgtgtccagagtcctgctctcactccatgtcatgttactggagttatcactccacttagctttcatctatttagagagggagaca
tccatgctgcacatcacattgctggatggagcccattacacatttccatactgtactgacagtgaccctggaagacagtagat
gatggatctgtatgaggccccatccaaggtgggaacactgatgcagttccaagctcctgggtccagctgccaagccttgag
tacactgcctttgttatcatctccataaaacattggccaacacaaccagattcagagatctgaatcttcatgattgatcatcgtt
ccatttcatattataaatgtttcataaataagaattcattatagctctatgaggtacaatgatatgtgtggacaaaatgaatgcattg
agtggaaattatgaactcagacttctgacacgttcataacctcaacacatgttttggaggagttcagctgtctcctgctcttaggagat
tccaaacacagttattcttaacctcttccatgctgtagagtagatccctcagtggtgagctgtgggactggaactctgac
cttgcctcaccattctgctcctcctcactcagacttggcttccactgtacttctgctctgtgagttcagcctttatcccgc
ataggtagcaccatattatgtgtcttctggcctggatggttagcagtggtgcactttactgacatattatcctagggacattattctc
caggatcaccagttgtccaaaatacagaatttctactctactgctataaaacttctttaaaatgtaacttttccatttcttctt
ccattcttcttctgagatgtcactggatcacacgtatgaactctttgattgatggtgctttaaacgaacaaggaactgcagaggt
gtttgccacactgactgcagatcccgtggacacctccctgggatttcatattcattgtaccatgcaatggtgcttgaacatcatgaa
taattctatttctagttttgatgctttccatacggtttactaattaacctgacacagactgtaccagggttatcctctctttacctata
aaacatgtcagctctggtatgtctgtaataagccatttaacaggagcagatgggatgtctcattgtgcttcaacatggacatacatg
atggctccagactgataacattttattgtctgtctatatacttcttttaataatttatagaaatgatataattttaaatttatttatta
aaaatttgtctglatgtatttgcctaaaaccacaaagagacggagactgacagtgatcttcaattcctgggtcacttcccaatggc
tacaaaagcttttagctggatgaggcaacagcctggttcttggaaattctgcccagtggtccgcatgggactacttgaacctcaccta
tggcctcccagggtgtgcttaggcaggaatctggaactggaaccaaactaggacgcagcactaagaatcctgacacgggagg
caggaatcccaggtcactccagtgctgctcctgtcaccacctatcagtcactgcatttaacaattgatttctcccttgggatt
cagttgctgagatcattcaacattaagaaacaggtcctaatcatatgtataattaatcttcaatttatttaagtagctatttatttattt
aaagtcaagtgtatagcaagagaggaagagaccaacagatggagggagaatcttctccacaggttccgtctgaatggctgt
aagactgaagatggctcagccagaaaccaggagcctggaacttctccagttctacaggggtggaaggggacaaacacttg
ggatggttagacagtgatcaacattgcactgcctacctgtgagttcaatcttggactgcatatataactaatgtcacatactat
gtgtctacgtgtaccgggattatttctgtagcatattgtgtccaggcagaccacgttcttattgacagaatgacctcttctaat
actgaataatattctatgtccaaatgcgaaagaatccattcagcttctatgcacactgcttattgtagactgaactattggaacaa
agctgcattacctgaacacaggatggcggctcaattgtgacacagagacttaccagtgcttgcactcaactcagagtcagatgat
tactagacacagagaagagaggggaacaggggaaggctgataccaggacacaaagcaccactcacaggaaggaataattct
gcccacaccgagtggtgactcagttcaccacaacacacgacaggtccacaaataatgagaactgaggtctccaggctgc
ccaactcagagtgattgaaaggaggtggaatgcgcatgacactgagttgatctgtgccatttctaccatgcacattccagtagc
actctataccccactaccatgtacagttctgagttcaggaacagttttaaacagtttaaatggtttaaactttaaagcaatggag
gaagaaggaaacaaaacatagttaaccttaaaaaatccactgacgtccttctctgtttaaagtgggcgacaggcatttggta
aaattggctaagtcccactaggacacccgtgttccatcacagaactgactgagcccagcctcacggcttcccacactttcct

Figure 13d Unit 4 15445bp Seq ID 192 (con't)

cataatgcatcatgggaaactgtagatgacgctacgagcaaaggagcccctaccaccaacgittgaattacagacacagtttctt
gctccagggttcagaaagactaactcctaggggtcataggcatttgggatgctggggctgtcaaccaaaggcagaaatctcc
cattgtcacctttctgtccatctctacatttctgtatctctgaccttctctctatcactgtaccaaggtcttcatatagatgaaaag
gaactaatggttattaaataaatgaggacatgatattgctaacattggatagaggggtacacacatataaatgaacaaggt
cattgccttcagcttttaaaattatgataacataaacattcatatctgaatcgtatcatagccatcacctacacaccaggaca
ccacgtctgcctgggccctgtcctgtctgaggcgtctgacatcatgcctgctatataggggcagcacatgcagatagggcctcc
ctctgccccatgaaaacctgcccagccctcacctgcagctctggcacaggagctccagccccagcactcccagggtgtccactc
agtgattgcactcaacacagatgctcaccatggagactgggctgcgctggcttctcctggctgctgtgctcaaaggtaatgatgg
ggaaggcggggcactgagtctgggagaggacgtgagtgagagacacagtgagtgtcagtgacagtgctgacctgtcgtc
tgtgttgcaggtgtccagtgtgaagtgcagctggtggagtctgggggaggcttggtagcctggcaggtccctgagactctcc
tgtgcagcctctggattcacctttgatgattatgcatgactgggtccggcaagctccagggaaggcctggagtgggtctcag
gtattagttggaatagtgtagcataggctatgcggactctgtgaaggccgattcaccatctccagagacaacgccaagaactc
cctgtatctgcaaatgaacagctctgagagctgaggacacggccttctgattactgtcaaaagatgacacagtgagggccctca
ggctgagcccagacacaagcctccctgcagggacgcgcggctccaccagggggcgcaaaagtctcactgagcgcagattct
ggttctgcacaggcacagaggagatgaagccctggttctctgtagggatggggtttctctcctgcaacagtttgagggaaga
attctctttatgattctgtccctgctactgaattcctgacatagagaagttgaaatgacaggagaagtcctatatgaaccaattcag
gttcagtggctccaggatcagagatgtgggtctatctgggtacaacgtgagtgttctcactctgagtcaggacaacgctctcag
ggcactgccgactttaggacaattttagatttcccttccgaccaggacagctgaggcagggttcttggctttataaatcagctatta
agtatctgtagcctgacaaaaagagcaagtgtgctgaactgaaccaaccttgactttactgtgggtgatccacacaaacttccga
tagactttatgctc gatttctatttcccttaaatcctggaacagctctgtggtccctgtgtgaacgtcacagcactcaggaatgtacttgc
agctcagctatgtctcaggctgagcttctgcaggcacctgggaagcctacagggacttctcctcctctaggtcctgggacctc
ctgtgggctccctgcatgaactcaggagggaatgccctggacatcagcagtcaggctcactgaatggaaaagtgtgggtg
tgcacttccagctgatctggttctgtctagtgtcgtgggaacctcgcactgctgtggtcaggagagcagaaaagtgggacac
acagtggctgtgactctggggctggagtccacatcacggggagctgtgtctgtggtccagcatgatgaccaatgatctgagg
ctgaaccacagctgcaggaagagccagtcaccagagaccattgtccagagtcctgctctcactccatgtctatgctgactggc
gtcatcaatccatttggagtcttagatcagttttagatgggagacaccaggctgcatacagagcttggacagagcccattaca
cattaccacacttgaactgacaatgcaccttgaagacagtagatgatggatctgttatgaggccccatctccaagggtgggaac
actgatgcagttccaagctcctgggttccagctgccaagcctgagtcactgccttattatcctcctataactttggcaaca
caacaccagattcagagatctgaattctatgatgggccattgttgcattcattcataaatatttataaataagaattgagtata
cctctatgaggtacaatgatatgtgtggacaaatgaatgcatattgagtggaaattatgaagtcagacttgatgacacattcataccg
caacacatgttttgggtggagtccagctgtctcctgcttttaggatttccactcacaatattcccttaagctctgtcaccatgctgtgca
ggagatccctcagtggtggtcctgtgggactgaaactctgacctgggtccaccatcactctgttctcctcactcagactctg
gtctcaccactgtacttctgctctgtgcttccagctttttccacataggtagatcacatattatgtgttctcctggcctggatgg
tgagcagagtgacttttactgacatattatcctagggcacattctccaggatcattaaagttgtccaaaattacagaatttctactc
tactgcttataaactccttttaaatgtaactttgtccatttcttcttctcatttcttccatgagatgctactggatcatatgtgtact
cttttaaatgaacaaggaactgcagaggtgttggcactgtgactgcagatcccgtgaacacctccctgggattcattcattgta
ccatgcaatggtgcttgaacatcatgtaataattctattctagttttgatgctttccataggtttactaattaacctgacacagactt
gtaccagggttatcctctctctacctccataaaaacatgcagctctggatgctgtgtaataagccattctaacaggagcagatggga
tgtctcgttgtcttcaacatggacatacatgatgggtcccaaatgatagcattttatgtctgtctatatacttcttttaataattata
gaaatgatataattttaaattatcattttatcagacctgtctttatgtattgtccaaaaccacagagagtgagactgacagtgatc
ttctaattcctgggtcacttccccaatggctacaaaagctcagctggatgaggcaacagcctgggtgcttggaaattctgtccatgtgt
ccgcatgggagcttggaaacctcacctacggcctcccagggtgtgcttagcaggaatctgaaactggaaccacactaggaca
cagcactaagcactcctacatgggaggeaggaatcccacaggctcactcaagtgtctcctgtcaccaccttatcctgctgtat
gtacacaattgatttctcccttgggattcagttgtctgtgtcattcaacattaacaaacaggctcctaatcatatgtataattaatctc

Figure 13d Unit 4 15445bp Seq ID 192 (con't)

aatgtatttaagtatctatttttttaaaagacagtgttatagcaacagaggaagagaccaacagatggaggagaattccttc
catttgctcacttccctgaatggctgcccaggctggggatggctaagcctaaagtcaggagcctggaactcctctagttccaca
gggctggaaggggacaaacatggtgacagtgatcaccattgcacttctacctctgtgagttcaactgtggactgcatataa
ctaattgcacttactatgtgtccacatgtacttgcattttcttttagttgtatgctccaggagaccacattcctaataacagaa
aacctccttcttaataactgaataatattccatgtccaaatgggaagaatgcattcactgttttatgcgcacttgccttggactga
agtattggaacaagctgcaacctgacacaggactgggggtaaattgtgacacagtgacttctaccagtgcttgagctgaact
cagagtcattggtactagacacaggggaaggggggcaacaggggacggctgataccaggacacaaagcaccactcacagg
aaggaataattctgcccacaccacagtgaggctgactcagttcaccacaacacagacagattccacaataactagaactaatgc
gtccaggctgcccactcagagtgattgaaaggaggtagaaatgtcatgacactgagttgatctgtgccatttctaccatgcac
attccaatagcactctatacccactaccatgtacagtggtctgtgttcaaaacagtttaaacatttaaacagttttaaacttaagg
caatggatgaataaggaaacaaacatagtaaccttaaaaaatgcacttgcagtccttcatctgtttaaagtgggtgacaggcat
taggagaaaattggctaagtcctcactagggacaccggagttcccgtaagagaactgactgagcccagcctcacggctccca
cacttttccctcataatgcacatgcccactagatgacactacaagaaaatgagcactaccactgacgttgaattacagacata
gtttcttgcctcagcgttcagaaaactaactcctagatgtcatagggcatttgggatgtctggggctgtcaacccaaaggcagaaat
ctcccattgtcacctttctgtccatctctacatttctgtatctctgaccccttctctctatcactgtcccaatgtcttcatatagatgat
aaggaactagtggtacttaataaatgaggacatgatattgtaacactggatatgagggtaaacacataataaatgaaacaa
ggtcattgccttcaacttttgaaattatgataacataaaaattcatatgatctgaatcatattatagtcacacctacaacccaggtc
acctaattctgccccaggccctatcctgtctgaggcatctgacccagggtgctatataggggcagcagatgcagatagccctc
cctctgcccataaaaaccagcccagccctcaccctgcagctctggcacaggagctccagcccaggactcccaggtgtccact
agtgatcacactcaacacagacgctcaccatggagactgggctgcgctggcttctcctggctgctgtgctcaaggtaatgatgg
ggaacgcggggcactgagctctgggagaggacgtgagtgagagacacagagagtgtagtgacagtgctgtgaccatgtcgtc
tgtgttgcagggtgtccagtgctcaggtgcagctgggtggagtctgggggaggcgtgggtccagcctgggaggtcctgagactctc
ctgtgcagcctctggattcacctcagtagctatggcatgactgggtccgagcctccaggcaaggggctggagtgggtggc
agttatatcatatgatggaagtaataaactatgcagactccgtgaagggccgattcaccatctccagagacaattcaagaaca
cgctgtatctgcaaatgaacagcctgagagctgaggacacggctgtgtattactgtgcgagagacacagtgaggggcccctcag
gtgagcccagacacaaacctcctgcagggccgtacgtctccaccaggaggcacacaagatgactgaatataatctgtgct
atgggtagggtgtagagggacaataagccctggtttcttttagggaattggggttctctccttaatacatttgaagaatggtct
actcttactgattctgtccctgccactgacttactgacggatagaaacttgaatagaaggaagaagtcctactgacaattcggg
ttcatgactgacaactatectcaccgcgatcagatatcatggggatctgtgaggacctctgagcttttccagctaaactgtgctg
gtgctcctcagggcgtgcccactctaggacaatttactttccctttatcctggagacaactgagccagggtctctgtgctgta
aacctcaactatttccctcttctgtcaaatgacctgtgtgcggaactgaattgacattttttaaactgtgggtgatccact
cactttctgcagaatgtggccaccttaactcctggaacagctgtggtccctgtgtgaacgtcacagcactcaggaatgacttgc
gctcagctgtgtctcagactgagcttctggaagcactgggaagcctacagggacttctcactccttaggtctgggacactcct
ggggggctccctgctgaactcggaggggaaatgactggacatcagcagtcaggatcactgaatggaaagtgtgggtg
tgcacttctagttgatctgattctgtgagtgttgggactccctgcacctgtgtggtcaggagagcagaaagtgggacacac
agtggctgtgactctggggctggagtccacatcacggggagctgtgtctgtggtccagcatgatgaccagtgctgactgacgt
gaatcacagctcaggaagagcaagtctcggagaccatgtgtccagagtcctgctctcactccatgtctatggtgactggggtc
atcaatgcatttggagacttccatcagtagtagaggagacacccaggctgcatacagagtgctggacacagcccatttga
cactttccagacaagtaactgacagtgaccctggaagtcagtagatgatggatgtgtatgaggccccatcacgaaggtggg
aacactaatgcagttcaagctcctgggtccagctgccaagctcagtcactggaattttatcatctccataaaaatattgaaa
atactgaaaagcactacaccagattcagagtggtgaatctcattttgatcatttaacatttctttacataagtgttgatataata
ccaaaacatagacataatgaggtacaatgatgtgtgataaataaatgatcaaatgagttttatgaagtcagacttattgacatg
ctcatcagaaaaccacatgttccatggtagaatgtcagttgtctgtgtttggaattctttaggcacaaatctccctcactgctgtg
acctgctgtacagtagatcctcagtgatggatcctgttggactggaactctgaccttgcctcaacgtctctctgctcctcccttg

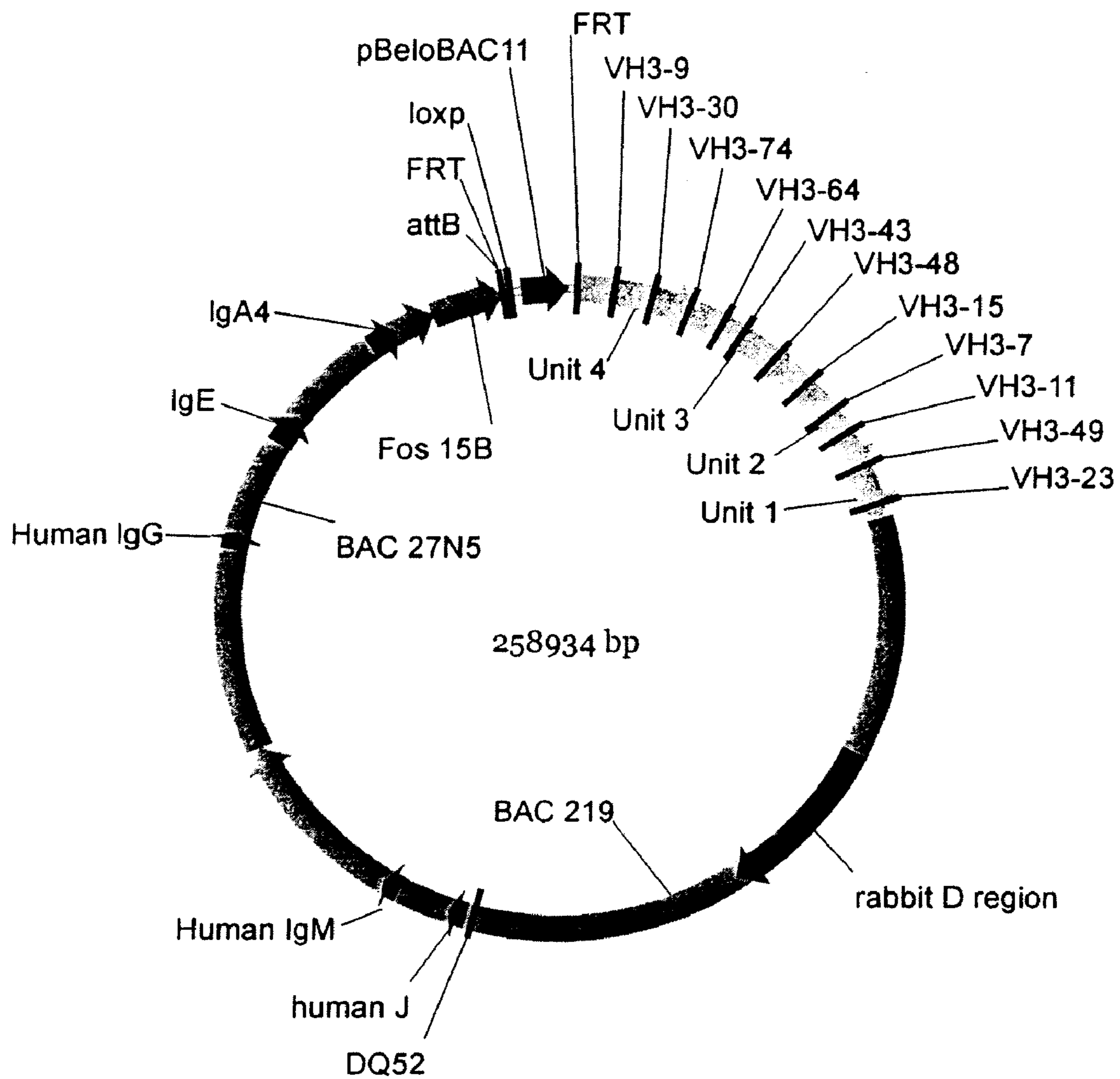
Figure 13d Unit 4 15445bp Seq ID 192 (con't)

ctcagacgttggcttcaccatcgtacttctgctctgtgacttcacctttatcccatgtatccagcatcacgtattattgtcttctg
tctgcatggttagcagagtgtacttaccctgacacattatccttggacattattctccaggatcatccgagttgtccaaaatacaga
atttctcatctgtactgccatataaacttctgttaaataataatttgcatttgtccattgttgattcttctcatgaatatttactgga
ttgatgtgtgaactcttgaatgatgctgcattagttgaacatggaagagcagaggtgttggccacattgattgcggttcccctgg
acacctcccctgggatttcatattccttctactcaaaattgggtgttgaagatcctgtgataattctgttcagtttgatgaattccataat
aatttacttgtttacctgactccattctgcaccagagttatctccagcttccctccacatggattgtcagctctggatttaggataata
ctcattctaacaggaaggatgggatgtctcattgtgcatctattagggacatgcatgatgggccaatatatcattttatattgtgcctt
tatacttttttaaaaagttaaagatactatttacttatttggatatttatttaccatattttttcaaaaaagcaaaagagactgagactga
cagtgatcttctaattcctgtcacttcccagatggatacaaaaagcttcagctgggttaggtgacagccatgtgcttgaattctgtc
cagttgtccacatggcagttacttgaacttccacttgcctcccagaagggttagacaggaatctgacactggaacgaaacta
ggacacagcactcagcactcctgacacgggagggcaggaatccctcaggtcactccagtgctgctcctgtcaccacctatcact
actgcatttaaggaattgattgcttccccttgggatcagtggtttagttcattcaacatttggaaataggctcctaaccatattgataat
taacttttatttttaagttttttttttttaaaggcagtggtatagcaagagagaaagctatcaacagatggagagagaagttc
tattcactggttcacttctgtaatggctgcaaaggctgggatggctgagcctgaagccaggagcctggaactccccagtttcc
cccagagctggaaggggacaaacttgggtcatgttacacagtgatcaccattgcacttctcccctctgtgagtcaatcttctg
gactgcatgtataactaatgtcacatactatgtgtccacatgtacctgcattatttctgttagcatattatgtcccaggctgaccaatga
tttccctaacgaccgaacaacctcttcttaacactgaataatattcattgcaaatgtaacagactgcattcactgttttaggcacatt
cactttatttagactgaactattgtgaacaaagctgcattacatgaaccaggactgtgggtgaattgtgacacagtgacttctatc
cagtgcttgagctgaactcagagtcattggttacttagacacaggggaagagggggaacacagggcagctgataccagggcacaa
agcactcacaggaaggaataatttgcacactacagtggtgactcagttcaccacaacctgacagattccatgaataat
gagaactgaggcctccaggcccctccaactcagagtgtaggaaaggaggtggaatgtgaatgacattcagttgatctgtgccc
atttctacaacacacagattccaatagcacagtagcacccttagcaagcacagtggttctgtgttcatgacgagtgtaataaagtc
aatggatgaataaggaaacaaaacatagttaacctaaaaagaatccacttgcagctttttatctgtttaaaagtgggagggcagc
taggacaaaattggcaagtcccacttagggacaccggtgttcccacagaatgaatcattcagccacacgactccccacacttt
ttctgataatgcattttggaaaactgtagatgacactacaagtgaatgagcccctaccactgacgtgggaaacaaaattgcagttttg
cgtccatgttttagaaaggctaaagctcctgggtgttaggcacttgggatgtttggggctgtcaatcaagggtggaatctcccctt
gtcacctttctgtccatctctacatttcttctctcacccttctcactgtcactattccaatgttattcaaatagttaaaatgaacc
atggttattttaaaatgaaggacatgataactggattattaaggatacacacatattaaaatgaacaaggctcattgccttcaatt
gtttaagcaacaatgataatgtaaagatttctatgatctgaatcctatcatagccatcaacctacaccacaggacaccacatctgcc
ctgggcacttctgtctgagcatcagaccccatgctgctatataggggcagcacaatgaaaacagggcctcccctctgccatg
aaaaccagcagggcctcaccctgcagctctggcacaggagctccagcctcaggactcccaggtgtccactcagtgatcacac
tcaacacagatgtcaccatggagactgggctgcgctggcttctcctggtcgtgctcaaggtaatgatggggaacgcggg
gcactgagctcggagaggacgtgagtgagagacacagagagtgctgctgacagtgctgacctgtctgtctgtttgcagg
tgtccagtgaggtgcagctggtggagtctgggggaggcttagttcagcctgggggtccctgagacttctctgtgcagcctctg
gattcacctcagtagctactggatgactgggtccccaagctccagggaaggggctgggtgggtctcacgtattaatagtga
tgggagtagcacaagctacgcggactccgtgaagggccgattcaccatctccagagacaacgccaagaacacgctgtatctgc
aaatgaacagctctgagagccgaggacacggctgtgtattactgtcaagagacacagtgaggggcccctcaggtgagcccag
acacaaacctccctgcaggggagtgcggctccaccagggtgcgcacaagacgcactgagcccagattctggccctgctctgat
gcagagggaaatgagccctggttctctcagtgatttgggtttgtctacagttttgcggcaagaattctacttcatgattctgtctg
ttccactgacttactgacttagagaagttttagtgacaggaagaagtcctgaatggacacattcaggttagtgaactatgacagt
ggtcaccagggtcagagatgtgagacaatcaggggacctcgtgagtggttccagctctgactcaggacagcgttccaggacact
gccgactataagacaatttttagtttcttcaaacccagagagagccgagtcagagcattgattttcaccttaattatataagtcag
acacatcctgtcggagtagcctgtgtgctgactgaaccaactgtctatttttaactgtgggtgttctgacttacttctacagactt

Figure 13d Unit 4 15445bp Seq ID 192 (con't)

Otataccccatctccatttcctttcatcctggaacagtctgtggccctgtgtgaacgtcacagcactcaggaatgtactgcagctc
agttgtgtctcaggctgagcttctgcaggcacctgtaccgttcgtatagcatacattatacgaagtatggccggccacgcgt

Figure 13e



HUMANIZED IMMUNOGLOBULIN LOCI**CROSS REFERENCE TO RELATED APPLICATIONS**

This application is a divisional of U.S. patent application Ser. No. 10/893,483 filed Jul. 15, 2004 now U.S. Pat. No. 7,585,668, which claims the benefit under 35 U.S.C. §119(e) of the priority of U.S. Provisional Application Ser. No. 60/487,733 filed on Jul. 15, 2003.

In accordance with 37 CFR 1.821(e), we hereby expressly incorporate herein by reference, in its entirety, the last-filed (filed Apr. 4, 2005) computer readable Sequence Listing, saved as "39691-0007A saved Apr. 4 2005.txt", date of creation Apr. 4, 2005, size 1,489 KB, submitted in U.S. application Ser. No. 10/893,483, filed Jul. 15, 2004.

FIELD OF THE INVENTION

The present invention concerns methods and means to produce humanized antibodies from transgenic non-human animals. The invention specifically relates to novel immunoglobulin heavy and light chain constructs, recombination and transgenic vectors useful in making transgenic non-human animals expressing humanized antibodies, transgenic animals, and humanized immunoglobulin preparations. The transgenic vectors contain humanized immunoglobulin loci, which are capable of undergoing gene rearrangement, gene conversion and hypermutation in transgenic non-human animals to produce diversified humanized antibodies, while leaving the endogenous regulatory and antibody production machinery of the non-human animals essentially intact. The humanized antibodies obtained have minimal immunogenicity to humans and are appropriate for use in the therapeutic treatment of human subjects.

BACKGROUND OF THE INVENTION

Antibodies are an important class of pharmaceutical products that have been successfully used in the treatment of various human diseases and conditions, such as cancer, allergic diseases, prevention of transplant rejection and host-versus-graft disease.

A major problem of antibody preparations obtained from animals is the intrinsic immunogenicity of non-human immunoglobulins in human patients. In order to reduce the immunogenicity of non-human antibodies, it has been shown that by fusing animal variable (V) region exons with human constant (C) region exons, a chimeric antibody gene can be obtained.

Humanized monoclonal antibodies have also been developed and are in clinical use. Humanized monoclonal antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in non-human animal, e.g. rodent, antibodies. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature*, 321: 522 (1986); Riechmann et al., *Nature*, 332: 323 (1988); Verhoeyen et al., *Science*, 239: 1534 (1988)), by substituting non-human animal, e.g. rodent, CDRs or CDR sequences for the corresponding sequences of a human monoclonal antibody.

It has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and genii-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line

mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., *Proc. Natl. Acad. Sci. USA*, 90: 2551 (1993); Jakobovits et al., *Nature*, 362: 255 (1993); Bruggemann et al., *Year in Immunol.*, 7: 33 (1993). While this genetic engineering approach resulted in the expression of human immunoglobulin polypeptides in genetically engineered mice, the level of human immunoglobulin expression is low. This may be due to species-specific regulatory elements in the immunoglobulin loci that are necessary for efficient expression of immunoglobulins. As demonstrated in transfected cell lines, regulatory elements present in human immunoglobulin genes may not function properly in non-human animals.

Indeed, several regulatory elements in immunoglobulin genes have been described. Of particular importance are enhancers downstream (3') of heavy chain constant regions and intronic enhancers in light chain genes. In addition, other, yet to be identified, control elements may be present in immunoglobulin genes. Studies in mice have shown that the membrane and cytoplasmic tail of the membrane form of immunoglobulin molecules play an important role in expression levels of human-mouse chimeric antibodies in the serum of mice homozygous for the human C γ 1 gene. Therefore, for the expression of heterologous immunoglobulin genes in animals it is desirable to replace sequences that contain enhancer elements and exons encoding transmembrane (M1 exon) and cytoplasmic tail (M2 exon) with sequences that are normally found in the animal in similar positions.

In addition to the issues raised by the potential immunogenicity of the non-human antibodies, the use of monoclonal antibodies in general, whether chimeric, humanized or human, is further limited by the fact that devastating diseases, such as cancer and infections with virulent pathogens, are difficult to treat by attacking one target, due to their complexity, multifactorial etiology and adaptivity. Monoclonal antibodies directed against singularly defined targets fail when those targets change, evolve and mutate. Thus, malignancies may gain resistance to standard monoclonal antibody therapies. A solution to this problem is the use of polyclonal antibodies, which have the ability to target and attack a plurality of evolving targets linked with complex diseases. Polyclonal antibodies also have the ability to neutralize bacterial and viral toxins, and direct immune responses to kill and eliminate pathogens.

Accordingly, there is a great clinical need for a new approach suitable for the large-scale production of high-titer, high-affinity, humanized poly- and monoclonal antibodies.

The introduction of human immunoglobulin genes into the genome of mice resulted in expression of a diversified human antibody repertoire in genetically engineered mice. In both mice and humans, primary antibody diversity is generated by gene rearrangement. This process results in the generation of many different recombined V(D)J segments encoding a large number of antibody molecules with different antigen binding sites. However, in other animals, like rabbits, pigs, cows and birds, primary antibody diversity is generated by substantially different mechanisms, namely templated mutations or gene conversion and non-templated mutations or hypermutation. For example, it is well established that in rabbit and chicken, VDJ rearrangement is very limited (almost 90% of immunoglobulin is generated with the 3' proximal VH1 element) and antibody diversity is generated by gene conversion and hypermutation. In contrast, mouse and human gene conversion occurs very rarely, if at all. Therefore, it is expected that in animals that diversify their primary antibody repertoire by gene conversion and hypermutation a genetic engineering approach based on gene rearrangement will result in animals

with low antibody titers and limited antibody diversity. Thus, the genetic engineering of large animals for the production of non-immunogenic antibody preparations for human therapy requires alternative genetic engineering strategies.

The production of humanized antibodies in transgenic non-human animals is described in PCT Publication No. WO 02/12437, published on Feb. 14, 2002, the disclosure of which is hereby expressly incorporated by reference in its entirety. WO 02/12437 describes genetically engineered non-human animals containing one or more humanized immunoglobulin loci which are capable of undergoing gene rearrangement and gene conversion in transgenic non-human animals, including animals in which antibody diversity is primarily generated by gene conversion to produce diversified humanized antibodies. The humanized antibodies obtained have minimal immunogenicity to humans and are appropriate for use in the therapeutic treatment of human subjects. It further describes novel nucleotide sequences from the 5' and 3' flanking regions of immunoglobulin heavy chain constant region segments of various non-human mammals, such as chickens, cows, sheep, and rabbits. Recombinant vectors in which human immunoglobulin heavy chain gene segments are flanked by sequences homologous to such 5' and 3' sequences are shown to be useful for replacing an immunoglobulin heavy chain gene segment of a non-human animal with the corresponding human immunoglobulin heavy chain gene segment.

SUMMARY OF THE INVENTION

In one aspect, the present invention concerns an isolated nucleic acid molecule comprising a human immunoglobulin gene segment, flanked by nucleotide sequences, wherein the flanking sequences are identical or different, and comprise at least about 20 contiguous nucleotides of a spacer sequence from an immunoglobulin heavy or light chain gene of an animal generating antibody diversity primarily by gene conversion and/or hypermutation, or from a consensus sequence of two or more of the spacer sequences.

In another aspect, the invention concerns an isolated nucleic acid molecule comprising a human immunoglobulin heavy or light chain constant region (C) gene segment, flanked by nucleotide sequences, wherein the flanking sequences are identical or different, and comprise at least about 20 contiguous nucleotides of a spacer sequence from an immunoglobulin heavy or light chain gene of a non-primate animal, or from a consensus sequence of two or more of the spacer sequences.

In a further aspect, the invention concerns an isolated nucleic acid molecule comprising a human immunoglobulin heavy or light chain gene segment, flanked by nucleotide sequences, wherein the flanking sequences are identical or different, and comprise at least about 20 contiguous nucleotides of a spacer sequence selected from the group consisting of SEQ ID NOS: 1 to 185 (Table 1), or from a consensus sequence of two or more of the spacer sequences.

In one embodiment, the flanking sequences comprise at least about 50 contiguous nucleotides of a spacer sequence.

In another embodiment, the human immunoglobulin gene segment is a heavy chain V, D, or J segment, where the V fragment may, for example be a member of the VH3, VH1, VH5, or VH4 family.

In a further embodiment, the human immunoglobulin gene segment is a light chain V or J segment, where the V segment may, for example be a κ light chain gene segment, such as V κ 1, V κ 3, or V κ 4, or a λ light chain segment, e.g. V λ 1, V λ 2 or V λ 3.

In a further embodiment, the non-primate animal which generates antibody diversity primarily by gene conversion and/or somatic hypermutation is, for example, rabbit, pig, bird, e.g. chicken, turkey, duck, or goose, sheep, goat, cow, horse or donkey, however, other non-primate animals, e.g. rodents are also specifically included within the scope of the invention.

In another aspect, the invention concerns a recombination vector comprising any of the foregoing nucleic acid molecules.

In yet another aspect, the invention concerns a transgenic vector comprising a humanized immunoglobulin (Ig) locus, wherein the humanized Ig locus is derived from an Ig locus or a portion of an Ig locus of a non-human animal, comprising multiple Ig segments wherein

- (a) at least one of the gene segments is a human Ig gene segment flanked by nucleotide sequences comprising at least about 20 contiguous nucleotides from a spacer sequence, or from a consensus sequence or two or more of such spacer sequences;
- (b) the gene segments are juxtaposed in an unrearranged, partially rearranged or fully rearranged configuration, and
- (c) the humanized Ig locus is capable of undergoing gene rearrangement, if necessary, as well as gene conversion and/or hypermutation, if the non-human animal is a gene converting animal, and producing a repertoire of humanized immunoglobulins in the non-human animal.

In a further embodiment, the humanized Ig heavy chain locus present in the transgenic vector comprises about 5 to 100 V gene segments, with at least one human V gene segment. In a specific embodiment, the humanized Ig heavy chain locus comprises more than one human V gene segments.

In another embodiment, the humanized Ig heavy chain locus present in the transgenic vector comprises about 5 to 25 D gene segments. In a specific embodiment, the humanized Ig heavy chain locus comprises one or several human D gene segments.

In yet another embodiment, the humanized Ig heavy chain locus present in the transgenic vector comprises about 1 to 10 J gene segments, with at least one human J gene segment. In a specific embodiment, the humanized Ig heavy chain locus comprises more than one human J gene segments.

In another embodiment, the humanized Ig heavy chain locus present in the transgenic vector comprises about 1-25 C region segments, with at least one human C region segment. In a specific embodiment, the humanized Ig heavy chain locus present in the transgenic vector comprises more than one human C gene segment.

In a still further embodiment, the humanized Ig locus present in the transgenic vector is a light chain locus of a non-human animal, and it comprises at least one V gene segment, at least one J gene segment and at least one constant (C) region gene segment, where at least one gene segment is selected from the group of human light chain V and J segments and human light chain C region segments. In a specific embodiment, the constant region gene segment is a human light chain constant region gene segment, which can, for example, be a C λ or C κ gene segment. In another embodiment, the humanized Ig light chain locus comprises two or more segments selected from human V and J segments and human C region segments. In a further embodiment, the humanized Ig light chain locus comprises at least one human V segment, at least one human J segment, and at least one human C region segment.

5

In a further embodiment, the humanized Ig light chain locus present in the transgenic vector comprises about 5-100 V gene segments, with at least one human V gene segment, wherein the human V gene segment is placed downstream to the 5-100 V gene segments of the non-human animal. In a specific embodiment, the human V gene segment is placed immediately 5' to a J gene segment in a rearranged configuration. In another embodiment, the humanized Ig light chain locus present in the transgenic vector comprises more than one human V gene segment.

In a still further embodiment, the humanized Ig light chain locus present in the transgenic vector comprises about 1-10 J gene segments, with at least one human J gene segment. In a specific embodiment, the humanized Ig light chain locus present in the transgenic vector comprises more than one human J gene segment.

In another embodiment, the humanized Ig light chain locus present in the transgenic vector comprises about 1-25 C region segments, with at least one human C region segment. In a specific embodiment, the humanized Ig light chain locus present in the transgenic vector comprises more than one human C gene segment.

In a still further embodiment, the humanized Ig locus present in the transgenic vector is a light chain locus of a non-human animal, and it comprises at least one

V gene segment, at least one J gene segment and at least one constant (C) region gene segment, where at least one gene segment is selected from the group of human light chain V and J segments and human light chain C region segments. In a specific embodiment, the constant region gene segment is a human light chain constant region gene segment, which can, for example, be a C λ or C κ gene segment. In another embodiment, the humanized Ig light chain locus comprises two or more segments selected from human V and J segments and human C region segments. In a further embodiment, the humanized Ig light chain locus comprises at least one human V segment, at least one human J segment, and at least one human C region segment.

In a different aspect, the invention concerns a nucleic acid molecule comprising two or more units consisting of, from 5' to 3' direction, a 5' nucleotide sequence, a human immunoglobulin sequence, and a 3' nucleotide sequence, wherein the 5' and 3' nucleotide sequences are identical or different, and comprise at least about 20 contiguous nucleotides from a spacer sequence separating the coding regions in a non-primate animal immunoglobulin heavy or light chain gene, or from a consensus sequence of two or more of the spacer sequences. In a specific embodiment, the spacer sequences are selected from within SEQ ID NOS: 1 to 185 (Table 1). In another particular embodiment, the 5' and/or 3' nucleotide sequences in all repetitive units of the nucleic acid molecule are identical. In another particular embodiment, the repetitive units of the nucleic acid molecule comprise at least two different 5' and/or 3' sequences. In a further embodiment, the 5' and 3' nucleotide sequence are different from each other, but all 5' and all 3' nucleotide sequences are identical.

In a further aspect, the invention concerns a method for making a transgenic vector comprising a humanized immunoglobulin (Ig) locus capable of producing a functional repertoire of humanized antibodies in a non-human animal, comprising:

- (a) obtaining a DNA fragment comprising an Ig locus or a portion thereof from the non-human animal which comprises at least one V gene segment, at least one J gene segment and at least one constant region gene segment; and

6

(b) integrating at least one human Ig gene segment into the DNA fragment of step (a) to produce a humanized Ig locus, wherein the human Ig gene segment flanked by nucleotide sequences comprising at least about 20 contiguous nucleotides from a spacer sequence separating the coding regions in a non-primate animal immunoglobulin heavy or light chain gene, or from a consensus sequence or two or more of such spacer sequences; wherein (i) the gene segments are juxtaposed in an unrearranged, partially rearranged or fully rearranged configuration, and (ii) the humanized Ig locus is capable of undergoing gene rearrangement, if necessary, and producing a repertoire of humanized immunoglobulins in the non-human animal.

The humanized Ig locus can be a humanized Ig heavy chain or light chain locus. In the case of a humanized Ig heavy chain locus the DNA fragment obtained in step (a) additionally comprises at least one D gene segment.

In another aspect, the invention concerns a transgenic animal comprising a humanized immunoglobulin locus described above, and methods for making such transgenic animals. In one embodiment, the transgenic animal comprises both a humanized immunoglobulin heavy chain locus and a humanized immunoglobulin light chain locus. In another embodiment, only one of the heavy and light chain loci present in the transgenic animal is humanized. In another embodiment, all of the V, D, J and C regions of at least one of the animal's immunoglobulin loci are humanized. In yet another embodiment, all of the V, D, J, and C region of the transgenic animals endogenous immunoglobulin loci are humanized.

In a further aspect, the invention concerns a B cell from the transgenic animals produced in accordance with the present invention.

In a still further aspect, the invention concerns a humanized immunoglobulin produced using a transgenic animal of the present invention, and an antibody preparation or a pharmaceutical composition comprising the humanized immunoglobulin.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic depiction of the rabbit immunoglobulin gene heavy chain locus.

FIGS. 2-4 show a comparison of rabbit heavy chain spacer sequences.

FIG. 5 is a schematic depiction of the rabbit immunoglobulin light chain locus.

FIG. 6 illustrates the building of an immunoglobulin gene V locus using human V_H and rabbit spacer elements.

FIG. 7: Insertion of two cassettes by homologous recombination using the red β γ -system. The upper cassette contains two restriction sites (FseI and AscI) flanking a gentamycin cassette. The lower cassette contains an inverted (i) and mutated (71) loxP-site, a FRT-site and a MluI-restriction site. After modification the BAC is digested with FseI and AscI.

FIG. 8 shows a humanized rabbit light chain locus (rLC3-B) based on the rabbit K1 light chain locus. Rabbit C κ 1 was replaced with human C κ . A human rearranged human V κ J κ was inserted. The synthetic human V κ J κ shares more than 80% sequence homology with rabbit V κ elements

FIG. 9a shows the sequence of a BAC clone comprising a chicken light chain genomic locus whose nucleotide sequence is shown in FIG. 9b (SEQ ID NO: 186).

FIG. 10: An outline showing the construction of a humanized immunoglobulin locus using chicken immunoglobulin spacer sequences and human V elements.

FIG. 11: An outline showing the construction of a humanized immunoglobulin locus using mouse or rabbit immunoglobulin spacer sequences and human V elements.

FIGS. 12a to 12c show a humanized light chain locus. A synthetic sequence (FIG. 12a, Unit 1, 12,235 bp, SEQ ID NO: 187) containing 17 human V pseudogenes and 18 chicken spacer sequences is shown in (a). A second synthetic sequence (FIG. 12b, Unit 2, 13,283 bp, SEQ ID NO: 188) containing a functional rearranged human VkJk gene fragment, 11 human V pseudogenes, 12 chicken spacer sequences and 2 introns is shown in (b). Units 1 and 2 were combined with a fragment derived from BAC 179L1 containing human Ck and rabbit intron and spacer sequences (FIG. 12c).

FIG. 13a-e show a humanized heavy chain locus. Four synthetic DNA fragments (Unit1-4, FIG. 13a-d, SEQ ID NOS: 189,190,191,192) consisting of human VH3 gene fragments and rabbit spacer and intron sequences were combined with parts of BAC 219D23, 27N5 and Fos15B as shown in (FIG. 13e).

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

“Antibodies” (Abs) and “immunoglobulins” (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

“Native antibodies and immunoglobulins” are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by covalent disulfide bond(s), while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains (Clothia et al., *J. Mol. Biol.* 186:651 (1985); Novotny and Haber, *Proc. Natl. Acad. Sci. U.S.A.* 82:4592 (1985)).

The term “variable” refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, connected by three CDRs. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an

antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

The term “monoclonal antibody” is used to refer to an antibody molecule synthesized by a single clone of B cells.

The term “polyclonal antibody” is used to refer to a population of antibody molecules synthesized by many clones of B cells. In a specific embodiment, polyclonal antibodies recognize several epitopes.

The terms “a humanized antibody” and “a humanized immunoglobulin”, as used herein, mean an immunoglobulin molecule comprising at least a portion of a human immunoglobulin polypeptide sequence (or a polypeptide sequence encoded by a human immunoglobulin gene segment). The humanized immunoglobulin molecules of the present invention can be isolated from a transgenic non-human animal engineered to produce humanized immunoglobulin molecules. Such humanized immunoglobulin molecules are less immunogenic to primates, especially humans, relative to non-humanized immunoglobulin molecules prepared from the animal or prepared from cells derived from the animal.

The term “non-human animal” as used herein includes, but is not limited to mammals, and includes, for example, non-human primates, rabbits, pigs, birds (e.g., chickens, turkeys, ducks, geese and the like), sheep, goats, cows, horses, and rodents (e.g. mice and rats). Preferred non-human animals are those animals which create antibody diversity substantially by gene conversion and/or somatic hypermutation, e.g., rabbit, pigs, birds (e.g., chicken, turkey, duck, goose and the like), sheep, goat, and cow. Particularly preferred non-human animals are rabbit and chicken.

The term “non-primate animal” as used herein includes, but is not limited to, mammals other than primates, including those listed above.

The phrase “animals which create antibody diversity substantially by gene conversion and/or hypermutation” is used to refer to animals in which the predominant mechanism of antibody diversification is gene conversion and/or hypermutation as opposed to gene rearrangement.

The term “Ig gene segment” as used herein refers to segments of DNA encoding various portions of an Ig molecule, which are present in the germline of animals and humans, and which are brought together in B cells to form rearranged Ig genes. Thus, Ig gene segments as used herein include V gene segments, D gene segments, J gene segments and C region gene segments.

The term “human Ig gene segment” as used herein includes both naturally occurring sequences of a human Ig gene segment, degenerate forms of naturally occurring sequences of a human Ig gene segment, as well as synthetic sequences that encode a polypeptide sequence substantially identical to the polypeptide encoded by a naturally occurring sequence of a human Ig gene segment. By “substantially” is meant that the degree of amino acid sequence identity is at least about 85%-95%. In a particular embodiment, the human Ig gene segment renders the immunoglobulin molecule non-immunogenic in humans.

A specific humanized immunoglobulin molecule of the present invention contains at least a portion of a human heavy or light chain variable region polypeptide sequence. Another specific immunoglobulin molecule contains at least a portion of a human heavy or light chain variable region polypeptide sequence, and at least a portion of a human constant domain polypeptide sequence.

By “a preparation of humanized antibodies” or “a humanized antibody preparation” is meant an isolated antibody product or a purified antibody product prepared from a trans-

genic non-human animal (e.g., serum, milk, or egg yolk of the animal) or from cells derived from a transgenic non-human animal (e.g., a B-cell or a hybridoma cell).

A humanized antibody preparation can be a preparation of polyclonal antibodies, which includes a repertoire of humanized immunoglobulin molecules. A humanized antibody preparation can also be a preparation of a monoclonal antibody.

The terms “antibody diversity” and “antibody repertoire” are used interchangeably, and refer to the total of all antibody specificities that an organism is capable of expressing.

The term “spacer sequence” is used herein to refer to any non-coding nucleotide sequence present in an immunoglobulin heavy or light chain gene. Thus, the term specifically includes intron sequences and any other non-coding sequences separating the coding regions within the V, D, J segments and C region segments in an immunoglobulin heavy chain gene, intron sequences and any other non-coding sequences separating the coding regions within the V and J segments and C region segments in an immunoglobulin light chain gene, as well as non-coding sequence flanking regulatory elements, such as enhancers, in an immunoglobulin heavy or light chain gene. In addition, non-coding sequences between exons encoding parts of a membrane-spanning helix and heavy and light chain enhancers are specifically included.

An Ig locus having the capacity to undergo gene rearrangement and gene conversion is also referred to herein as a “functional” Ig locus, and the antibodies with a diversity generated by a functional Ig locus are also referred to herein as “functional” antibodies or a “functional” repertoire of antibodies.

B. Relevant Literature

Regulatory elements in immunoglobulin genes have been described by Bradley et al. (1999), *Transcriptional enhancers and the evolution of the IgH locus*; Lauster, R. et al., *Embo J* 12: 4615-23 (1993); Volgna et al., *J Immunol* 165:6400 (2000); Hole et al., *J Immunol* 146:4377 (1991).

Antibody diversification by gene conversion in chicken and rabbit has been described by Bucchini et al., *Nature* 326: 409-11 (1987); Knight et al., *Advances in Immunology* 56: 179-218 (1994); Langman et al., *Res Immunol* 144: 422-46 (1993). The generation of mice expressing human-mouse chimeric antibodies has been described by Pluschke et al., *Journal of Immunological Methods* 215: 27-37 (1998). The generation of mice expressing human-mouse chimeric antibodies with mouse derived membrane and cytoplasmic tails has been described by Zou et al., *Science* 262: 1271-1274 (1993); Zou et al. *Curr Biol* 4: 1099-1103. The generation of mice expressing human immunoglobulin polypeptides has been described by Bruggemann et al. *Curr Opin Biotechnol* 8(4): 455-8 (1997); Lonberg et al. *Int Rev Immunol* 13(1):65-93 (1995); Neuberger et al., *Nature* 338: 350-2 (1989). Generation of transgenic mice using a BAC clone has been described by Yang et al., *Nat Biotechnol* 15: 859-65 (1997). The generation of cows expressing human antibodies has been described by Kuroiwa et al., *Nature Biotech* 20(9): 889-894 (2002).

The generation of transgenic rabbits has been described by Fan, J. et al., *Pathol Int* 49: 583-94 (1999); Brem et al., *Mol Reprod Dev* 44: 56-62 (1996). Rabbits with impaired immunoglobulin expression have been described by McCartney-Francis et al., *Mol Immunol* 24: 357-64 (1987); Allegrucci, et al., *Eur J Immunol* 21: 411-7 (1991).

The production of transgenic chicken has been described by Sherman et al., *Nature Biotech* 16:1050-1053 (1998); Etches et al., *Methods in Molecular Biology* 62: 433-450; Pain et al., *Cells Tissues Organs* 165(3-4): 212-9 (1999);

Sang, H., “Transgenic chickens—methods and potential applications”, *Trends Biotechnol* 12:415 (1994); and in WO2004003157, “Gene regulation in transgenic animals using a transposon based vector”; and in WO 200075300, “Introducing a nucleic acid into an avian genome, useful for transfecting avian blastodermal cells for producing transgenic avian animals with the desired genes, by directly introducing the nucleic acid into the germinal disc of the egg”.

A gammaglobulinemic chicken have been described by Frommel et al., *J Immunol* 105(1): 1-6 (1970); Benedict et al., *Adv Exp Med Biol* 1977; 88(2): 197-205.

The cloning of animals from cells has been described by T. Wakayama et al., *Nature* 1998; 394:369-374; J. B. Cibelli et al., *Science* 280:1256-1258 (1998); J. B. Cibelli et al., *Nature Biotechnology* 1998; 16:642-646; A. E. Schnieke et al., *Science* 278: 2130-2133 (1997); K. H. Campbell et al., *Nature* 380: 64-66 (1996), Kuroiwa et al., *Nature Genetics* 2004, Jun. 6. Nuclear transfer cloning of rabbits has been described by Stice et al., *Biology of Reproduction* 39: 657-664 (1988), and Challah-Jacques et al., *Cloning and Stem Cells* 8(4):295-299 (2003).

Production of antibodies from transgenic animals is described in U.S. Pat. Nos. 5,814,318, 5,545,807 and 5,570,429. Homologous recombination for chimeric mammalian hosts is exemplified in U.S. Pat. No. 5,416,260. A method for introducing DNA into an embryo is described in U.S. Pat. No. 5,567,607. Maintenance and expansion of embryonic stem cells is described in U.S. Pat. No. 5,453,357.

The mechanisms involved in the diversification of the antibody repertoire in pigs, sheep and cows are reviewed in Butler, J. E. (1998), “Immunoglobulin diversity, B-cell and antibody repertoire development in large farm animals”, *Rev Sci Tech* 17:43. Antibody diversification in sheep is described in Reynaud, C. A., C. Garcia, W. R. Hein, and J. C. Weill (1995), “Hypermutation generating the sheep immunoglobulin repertoire is an antigen-independent process”, *Cell* 80:115; and Dufour, V., S. Malinge, and F. Nau. (1996), “The sheep Ig variable region repertoire consists of a single VH family,” *J Immunol* 156:2163.

C. Detailed Description

Immunoglobulin heavy and light chain genes comprise several segments encoded by individual genes and separated by intron sequences. Thus genes for the human immunoglobulin heavy chain are found on chromosome 14. The variable region of the heavy chain (VH) comprises three gene segments: V, D and J segments, followed by multiple genes coding for the C region. The V region is separated from the C region by a large spacer, and the individual genes encoding the V, D and J segments are also separated by spacers.

There are two types of immunoglobulin light chains: κ and λ . Genes for the human κ light chain are found on chromosome 2 and genes for the human λ light chain are found on chromosome 22. The variable region of antibody light chains includes a V segment and a J segment, encoded by separate gene segments. In the germline configuration of the κ light chain gene, there are approximately 100-200 V region genes in linear arrangement, each gene having its own leader sequence, followed by approximately 5 J gene segments, and C region gene segment. All V regions are separated by introns, and there are introns separating the V, J and C region gene segments as well.

The immune system’s capacity to protect against infection rests in a genetic machinery specialized to create a diverse repertoire of antibodies. Antibody-coding genes in B cells are assembled in a manner that allows to countless combinations of binding sites in the variable (V) region. It is estimated that more than 10^{12} possible binding structures arise from such

mechanisms. In all animals, including humans, the antibody-making process begins by recombining variable (V), diversity (D) and joining (J) segments of the immunoglobulin (Ig) locus. Following this step, depending on the animal species, two general mechanisms are used to produce the diverse binding structures of antibodies.

In some animals, such as human and mouse, there are multiple copies of V, D and J gene segments on the immunoglobulin heavy chain locus, and multiple copies of V and J gene segments on the immunoglobulin light chain locus. Antibody diversity in these animals is generated primarily by gene rearrangement, i.e., different combinations of gene segments to form rearranged heavy chain variable region and light chain variable region. In other animals (e.g., rabbit, birds, e.g., chicken, goose, and duck, sheep, goat, and cow), however, gene rearrangement plays a smaller role in the generation of antibody diversity. For example, in rabbit, only a very limited number of the V gene segments, most often the V gene segments at the 3' end of the V-region, is used in gene rearrangement to form a contiguous VDJ segment. In chicken, only one V gene segment (the one adjacent to the D region, or "the 3' proximal V gene segment"), one D segment and one J segment are used in the heavy chain rearrangement; and only one V gene segment (the 3' proximal V segment) and one J segment are used in the light chain rearrangement. Thus, in these animals, there is little diversity among initially rearranged variable region sequences resulting from junctional diversification. Further diversification of the rearranged Ig genes is achieved by gene conversion, a process in which short sequences derived from the upstream V gene segments replace short sequences within the V gene segment in the rearranged Ig gene. Additional diversification of antibody sequences may be generated by hypermutation.

Immunoglobulins (antibodies) belong into five classes (IgG, IgM, IgA, IgE, and IgD, each with different biological roles in immune defense. The most abundant in the blood and potent in response to infection is the IgG class. Within the human IgG class, there are four sub-classes (IgG1, IgG2, IgG3 and IgG4 isotypes) determined by the structure of the heavy chain constant regions that comprise the Fc domain. The F(ab) domains of antibodies bind to specific sequences (epitopes) on antigens, while the Fc domain of antibodies recruits and activates other components of the immune system in order to eliminate the antigens.

Antibodies have been used successfully as therapeutics since the 1890s when it was found that polyclonal antiserum taken from animals could treat life-threatening infections in humans. A significant advance in antibody research occurred with the development of methods for the recombinant production of antibodies, followed by the development of antibody humanization techniques and method for making fully human monoclonal antibodies in non-human animals.

As a result, chimeric, humanized and human monoclonal antibodies have recently emerged as an important class of pharmaceutical products. While monoclonal antibody-based drugs are very effective in treating diseases when blocking a particular target (e.g. receptor or ligand) certain devastating diseases, such as cancer and infections with virulent pathogens, may be difficult to treat due to their complexity, multifactorial etiology and adaptivity. Monoclonal antibodies address singularly defined targets that change, evolve and mutate during the spread of diseases throughout a population or within an individual. Such adaptive evolution is the bane of mono-specific drugs (e.g. monoclonal antibodies), which are quickly circumvented by resistant strains. Examples abound of bacterial and viral resistance to high-potency antibiotics,

and malignant cancers that develop resistance to standard anticancer drugs, such as monoclonal antibody therapies.

In contrast, polyclonal antibodies have the ability to bind and eliminate a plurality of evolving targets linked with complex diseases. By binding multiple antigens, polyclonal antibodies saturate the target and retain activity even in the event of antigen mutation. Following this, through a cascade of signals, polyclonal antibodies induce a potent immune response to eliminate the target antigen, pathogen or cell. These properties make polyclonal antibodies ideal for treating infectious diseases and cancer.

So far, the use of polyclonal antibodies has been severely limited by either supply problems or unwanted reactions to non-human proteins.

The present invention provides a new humanization approach, based on selective humanization the immunoglobulin-coding elements of the immunoglobulin (Ig) translocus. The creation of such human-animal translocus allows for the creation of transgenic animals that express diversified, high-affinity humanized (polyclonal) antibodies in high yields.

As a first step, the genomic loci for non-human, including non-primate, immunoglobulin heavy and light chains are identified and sequenced. For example, as part of the present invention, genomic sequences for rabbit and chicken immunoglobulin heavy and light chains were determined, and are shown in FIGS. 1, 5, and 9.

Analysis of the rabbit Ig heavy chain genomic locus has shown that the immunoglobulin heavy chain variable region (Vh) contains numerous genes, including functional genes and non-functional pseudogenes. Alignment of 18 Vh genes has revealed a high degree (80-90%) sequence identity among rabbit heavy chain variable region gene sequences (Vh1-Vh18). The rabbit heavy chain variable region genes have been found to share highest homology with the Vh3 group of the human heavy chain variable region genes. Specifically, sequence comparison of the rabbit Vh1-a2 gene with the human Vh3-23 sequences has revealed 72.8% sequence identity.

In addition, the non-coding (e.g. intron) sequences separating the rabbit heavy chain variable region gene sequences were analyzed. FIGS. 2-4 show a comparison of rabbit heavy chain intron sequences. It has been found that such intron sequences fall into two groups, and are highly conserved. Especially members of the Group 1 introns show a surprisingly high (80-90%) sequence identity.

Similar findings were made by analysis of rabbit immunoglobulin light chain variable region genomic sequences. In particular, analysis of the rabbit immunoglobulin light chain locus has shown that the light chain variable region (Vl) region contains numerous gene segments, which show a high degree (80-90% sequence identity). It has further been found that the rabbit light chain variable region (Vk) exhibits high homology with the Vk1 group of the human light chain variable region gene sequences. Most Vk sequences have been found to be functional and highly conserved. Unlike in the rabbit heavy chain variable region genes, in the rabbit light chain variable region genes the intron sequences have been found to be heterogeneous.

Similar studies with chicken immunoglobulin heavy and light chain genomic sequences provide analogous results.

In one aspect, the present invention provides spacer sequences, which separate the coding regions in a non-primate animal heavy or light chain gene. In one embodiment, the present invention provides spacer sequences from the heavy and light chain genes of animals which create antibody diversity substantially by gene conversion, including, for

example, rabbit and chicken. Such spacer sequences are then used to flank human immunoglobulin heavy or light chain gene segments used in the process of creating a humanized immunoglobulin locus.

The spacer sequences typically comprise at least about 20 nucleotides, or at least about 30 nucleotides, or at least about 40 nucleotides, or at least about 50 nucleotides, and typically are between about 20 and about 10000 nucleotides in length. The spacer sequences may contain a contiguous stretch of nucleotides of appropriate length from a naturally occurring intron sequence in a non-human (e.g. non-primate) animal, or may include an artificial sequence, which may, for example, be a consensus sequence of two or more naturally occurring intron sequences.

The spacer sequences may comprise at least about 20 (30, 40, 50, etc. up to 1000 in 10-nucleotide increments) contiguous nucleotides from a sequence selected from the group consisting of SEQ ID NOS 1 to 185 (Table 1), or from a consensus sequence of two or more of such sequences. It is possible, but not necessary, to separate human heavy or light chain sequences (e.g. V, D, J, C region sequences) used for humanization by spacer sequences that separate the corresponding regions within the genomic sequence of the non-human (non-primate) animal the immunoglobulin of which is humanized.

In general, the humanization of an immunoglobulin (Ig) locus in a non-human animal involves the integration of one or more human Ig gene segments into the animal's genome to create humanized immunoglobulin loci. Thus, creation of a humanized Ig heavy chain locus involves the integration of one or more V and/or D and/or J segments, and/or C region segments into the animal's genome. Similarly, the creation of a humanized Ig light chain locus involves the integration of one or more V and/or J segments, and/or C region segments into the animal's genome.

Depending upon the approach used, the human Ig gene segment(s) can be integrated at the chromosomal location where the endogenous Ig locus of the animal ordinarily resides, or at a different locus of the animal. Regardless of the chromosomal location, the humanized Ig locus of the present invention has the capacity to undergo gene rearrangement and gene conversion and hypermutation in the non-human animal, thereby producing a diversified repertoire of humanized Ig molecules. An Ig locus having the capacity to undergo gene rearrangement and gene conversion is also referred to as a "functional" Ig locus and the antibodies with a diversity generated by a functional Ig locus are also referred to as "functional" antibodies or a "functional" repertoire of antibody molecules.

In a further aspect, the invention provides nucleic acid molecules comprising a human Ig gene segment, flanked by nucleotide sequences which comprise at least about 20 contiguous nucleotides from a spacer sequence separating the coding regions in a non-primate animal Ig heavy or light chain gene, or from a consensus sequence of two or more of such spacer sequences. The flanking sequences just as the spacer sequence-derived sections within the flanking sequences can be identical or different. The contiguous nucleotides derived from a spacer sequence or from a consensus sequence of two or more spacer sequences can be fused directly to the human Ig gene segment. Alternatively, there might be an intervening sequence between the human Ig gene segment and at least one of the spacer-originating nucleotide sequences. Thus, for example, a flanking sequence at the 5' end of a human V gene segment may include a promoter region, which is linked directly to the human V gene segment,

and separates it from the spacer-sequence derived nucleotide stretch of at least 20 nucleotides.

In yet another aspect, the invention concerns a humanized Ig heavy chain locus in which human heavy chain V, D and/or J gene segments and/or C region segments are present in the same configuration as in the original non-human animal immunoglobulin gene, and separated by sequences including at least about 20 contiguous nucleotides from an intron sequence separating the coding regions in a non-primate animal Ig heavy or light chain gene. In another embodiment, the present invention provides a humanized light chain locus in which human light chain C region segments and/or J gene segments and/or V region segments are separated by non-human animal (e.g. non-primate) intron sequences in the same configuration as in the original non-human animal immunoglobulin gene. In a particular embodiment, the spacer sequences are designed based on non-coding, e.g. intron sequences of the non-human (non-primate) animal. In one embodiment, the spacers may retain the appropriate non-coding sequences from the non-human (non-primate) animal. Alternatively, in order to simplify the construct, a consensus sequence, designed based upon the highly homologous non-coding (intron) sequences may be designed, and used as a uniform spacer sequence for the preparation of multiple human heavy or light chain gene segments.

The invention specifically provides isolated nucleic acid sequences and vectors useful in the construction of humanized immunoglobulin loci.

In one embodiment, DNA fragments containing an Ig locus to be humanized are isolated from animals which generate antibody diversity by gene conversion, e.g., rabbit and chicken. Such large DNA fragments can be isolated by screening a library of plasmids, cosmids, yeast artificial chromosomes (YACs) or bacterial artificial chromosomes (BACs), and the like, prepared from the genomic DNA of the non-human, e.g. non-primate animal. An entire animal C-region can be contained in one plasmid or cosmid clone which is subsequently subjected to humanization. YAC clones can carry DNA fragments of up to 2 megabases, thus an entire animal heavy chain locus or a large portion thereof can be isolated in one YAC clone, or reconstructed to be contained in one YAC clone. BAC clones are capable of carrying DNA fragments of smaller sizes (about 150-450 kb). However, multiple BAC clones containing overlapping fragments of an Ig locus can be separately humanized and subsequently injected together into an animal recipient cell, wherein the overlapping fragments recombine in the recipient animal cell to generate a continuous Ig locus.

Human Ig gene segments can be integrated into the Ig locus on a vector (e.g., a BAC clone) by a variety of methods, including ligation of DNA fragments, or insertion of DNA fragments by homologous recombination. Integration of the human Ig gene segments is done in such a way that the human Ig gene segment is operably linked to the host animal sequence in the transgene to produce a functional humanized Ig locus, i.e., an Ig locus capable of gene rearrangement and gene conversion and hypermutation which lead to the production of a diversified repertoire of humanized antibodies.

In one embodiment, human Ig gene segments can be integrated into the Ig locus by homologous recombination. Homologous recombination can be performed in bacteria, yeast and other cells with a high frequency of homologous recombination events. For example, a yeast cell is transformed with a YAC containing an animal's Ig locus or a large portion thereof. Subsequently, such yeast cell is further transformed with a recombination vector as described hereinabove, which carries a human Ig gene segment linked to a 5'

flanking sequence and a 3' flanking sequence. The 5' and the 3' flanking sequences in the recombination vector are homologous to those flanking sequences of the animal Ig gene segment on the YAC. As a result of a homologous recombination, the animal Ig gene segment on the YAC is replaced with the human Ig gene segment. Alternatively, a bacterial cell such as *E. coli* is transformed with a BAC containing an animal's Ig locus or a large portion thereof. Such bacterial cell is further transformed with a recombination vector which carries a human Ig gene segment linked to a 5' flanking sequence and a 3' flanking sequence. The 5' and the 3' flanking sequences in the recombination vector mediate homologous recombination and exchange between the human Ig gene segment on the recombination vector and the animal Ig gene segment on the BAC. Humanized YACs and BACs can be readily isolated from the cells and used in making transgenic animals.

In a further aspect of the present invention, methods of making transgenic animals capable of producing humanized immunoglobulins are provided.

According to the present invention, a transgenic animal capable of making humanized immunoglobulins are made by introducing into a recipient cell or cells of an animal one or more of the transgenic vectors described herein above which carry a humanized Ig locus, and deriving an animal from the genetically modified recipient cell or cells.

The recipient cells may, for example, be from non-human animals which generate antibody diversity by gene conversion and/or hypermutation, e.g., bird (such as chicken), rabbit, cows and the like. In such animals, the 3' proximal V gene segment is preferentially used for the production of immunoglobulins. Integration of a human V gene segment into the Ig locus on the transgene vector, either by replacing the 3' proximal V gene segment of the animal or by being placed in close proximity of the 3' proximal V gene segment, results in expression of human V region polypeptide sequences in the majority of immunoglobulins. Alternatively, a rearranged human V(D)J segment may be inserted into the J locus of the immunoglobulin locus on the transgene vector.

The transgenic vectors containing a humanized Ig locus is introduced into the recipient cell or cells and then integrated into the genome of the recipient cell or cells by random integration or by targeted integration.

For random integration, a transgenic vector containing a humanized Ig locus can be introduced into an animal recipient cell by standard transgenic technology. For example, a transgenic vector can be directly injected into the pronucleus of a fertilized oocyte. A transgenic vector can also be introduced by co-incubation of sperm with the transgenic vector before fertilization of the oocyte. Transgenic animals can be developed from fertilized oocytes. Another way to introduce a transgenic vector is by transfecting embryonic stem cells and subsequently injecting the genetically modified embryonic stem cells into developing embryos. Alternatively, a transgenic vector (naked or in combination with facilitating reagents) can be directly injected into a developing embryo. Ultimately, chimeric transgenic animals are produced from the embryos which contain the humanized Ig transgene integrated in the genome of at least some somatic cells of the transgenic animal.

In a particular embodiment, a transgene containing a humanized Ig locus is randomly integrated into the genome of recipient cells (such as fertilized oocyte or developing embryos) derived from animal strains with an impaired expression of endogenous immunoglobulin genes. The use of such animal strains permits preferential expression of immunoglobulin molecules from the humanized transgenic Ig

locus. Examples for such animals include the Alicia and Basilea rabbit strains, as well as Agammaglobinemic chicken strain, as well as immunoglobulin knock-out mice. Alternatively, transgenic animals with humanized immunoglobulin transgenes or loci can be mated with animal strains with impaired expression of endogenous immunoglobulins. Offspring homozygous for an impaired endogenous Ig locus and a humanized transgenic Ig locus can be obtained.

For targeted integration, a transgenic vector can be introduced into appropriate animal recipient cells such as embryonic stem cells or already differentiated somatic cells. Afterwards, cells in which the transgene has integrated into the animal genome and has replaced the corresponding endogenous Ig locus by homologous recombination can be selected by standard methods. See for example, Kuroiwa et al, Nature Genetics 2004, Jun. 6. The selected cells may then be fused with enucleated nuclear transfer unit cells, e.g. oocytes or embryonic stem cells, cells which are totipotent and capable of forming a functional neonate. Fusion is performed in accordance with conventional techniques which are well established. Enucleation of oocytes and nuclear transfer can also be performed by microsurgery using injection pipettes. (See, for example, Wakayama et al., Nature (1998) 394:369.) The resulting egg cells are then cultivated in an appropriate medium, and transferred into synchronized recipients for generating transgenic animals. Alternatively, the selected genetically modified cells can be injected into developing embryos which are subsequently developed into chimeric animals.

Further, according to the present invention, a transgenic animal capable of producing humanized immunoglobulins can also be made by introducing into a recipient cell or cells, one or more of the recombination vectors described herein above, which carry a human Ig gene segment, linked to 5' and 3' flanking sequences that are homologous to the flanking sequences of the endogenous Ig gene segment, selecting cells in which the endogenous Ig gene segment is replaced by the human Ig gene segment by homologous recombination, and deriving an animal from the selected genetically modified recipient cell or cells.

Similar to the target insertion of a transgenic vector, cells appropriate for use as recipient cells in this approach include embryonic stem cells or already differentiated somatic cells. A recombination vector carrying a human Ig gene segment can be introduced into such recipient cells by any feasible means, e.g., transfection. Afterwards, cells in which the human Ig gene segment has replaced the corresponding endogenous Ig gene segment by homologous recombination, can be selected by standard methods. These genetically modified cells can serve as nuclei donor cells in a nuclear transfer procedure for cloning a transgenic animal. Alternatively, the selected genetically modified embryonic stem cells can be injected into developing embryos which can be subsequently developed into chimeric animals.

Transgenic animals produced by any of the foregoing methods form another embodiment of the present invention. The transgenic animals have at least one, i.e., one or more, humanized Ig loci in the genome, from which a functional repertoire of humanized antibodies is produced.

In a specific embodiment, the present invention provides transgenic rabbits having one or more humanized Ig loci in the genome. The transgenic rabbits of the present invention are capable of rearranging and gene converting the humanized Ig loci, and expressing a functional repertoire of humanized antibodies.

In another specific embodiment, the present invention provides transgenic chickens having one or more humanized Ig

loci in the genome. The transgenic chickens of the present invention are capable of rearranging and gene converting the humanized Ig loci, and expressing a functional repertoire of humanized antibodies. In another specific embodiment, the present invention provides transgenic mice with one or more humanized V regions in the genome. The humanized V region comprises at least two human V gene segments flanked by non-human spacer sequences. The transgenic mice are capable of rearranging the human V elements and expressing a functional repertoire of antibodies.

Once a transgenic non-human animal capable of producing diversified humanized immunoglobulin molecules is made, humanized immunoglobulins and humanized antibody preparations against an antigen can be readily obtained by immunizing the animal with the antigen. A variety of antigens can be used to immunize a transgenic host animal. Such antigens include, without limitation, microorganisms, e.g. viruses and unicellular organisms (such as bacteria and fungi), alive, attenuated or dead, fragments of the microorganisms, or antigenic molecules isolated from the microorganisms.

Exemplary bacterial antigens for use in immunizing an animal include purified antigens from *Staphylococcus aureus* such as capsular polysaccharides type 5 and 8, recombinant versions of virulence factors such as alpha-toxin, adhesin binding proteins, collagen binding proteins, and fibronectin binding proteins. Exemplary bacterial antigens also include an attenuated version of *S. aureus*, *Pseudomonas aeruginosa*, *enterococcus*, *enterobacter*, and *Klebsiella pneumoniae*, or culture supernatant from these bacteria cells. Other bacterial antigens which can be used in immunization include purified lipopolysaccharide (LPS), capsular antigens, capsular polysaccharides and/or recombinant versions of the outer membrane proteins, fibronectin binding proteins, endotoxin, and exotoxin from *Pseudomonas aeruginosa*, *enterococcus*, *enterobacter*, and *Klebsiella pneumoniae*.

Exemplary antigens for the generation of antibodies against fungi include attenuated version of fungi or outer membrane proteins thereof, which fungi include, but are not limited to, *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, and *Cryptococcus neoformans*.

Exemplary antigens for use in immunization in order to generate antibodies against viruses include the envelop proteins and attenuated versions of viruses which include, but are not limited to respiratory syncytial virus (RSV) (particularly the F-Protein), Hepatitis C virus (HCV), Hepatitis B virus (HBV), cytomegalovirus (CMV), EBV, and HSV.

Therapeutic antibodies can be generated for the treatment of cancer by immunizing transgenic animals with isolated tumor cells or tumor cell lines; tumor-associated antigens which include, but are not limited to, Her-2-neu antigen (antibodies against which are useful for the treatment of breast cancer); CD19, CD20, CD22 and CD53 antigens (antibodies against which are useful for the treatment of B cell lymphomas), (3) prostate specific membrane antigen (PMSA) (antibodies against which are useful for the treatment of prostate cancer), and 17-1A molecule (antibodies against which are useful for the treatment of colon cancer).

The antigens can be administered to a transgenic host animal in any convenient manner, with or without an adjuvant, and can be administered in accordance with a predetermined schedule.

After immunization, serum or milk from the immunized transgenic animals can be fractionated for the purification of pharmaceutical grade polyclonal antibodies specific for the antigen. In the case of transgenic birds, antibodies can also be made by fractionating egg yolks. A concentrated, purified

immunoglobulin fraction may be obtained by chromatography (affinity, ionic exchange, gel filtration, etc.), selective precipitation with salts such as ammonium sulfate, organic solvents such as ethanol, or polymers such as polyethyleneglycol.

For making a monoclonal antibody, spleen cells are isolated from the immunized transgenic animal and used either in cell fusion with transformed cell lines for the production of hybridomas, or cDNAs encoding antibodies are cloned by standard molecular biology techniques and expressed in transfected cells. The procedures for making monoclonal antibodies are well established in the art. See, e.g., European Patent Application 0 583 980 A1 ("Method For Generating Monoclonal Antibodies From Rabbits"), U.S. Pat. No. 4,977,081 ("Stable Rabbit-Mouse Hybridomas And Secretion Products Thereof"), WO 97/16537 ("Stable Chicken B-cell Line And Method of Use Thereof"), and EP 0 491 057 B1 ("Hybridoma Which Produces Avian Specific Immunoglobulin G"), the disclosures of which are incorporated herein by reference. In vitro production of monoclonal antibodies from cloned cDNA molecules has been described by Andris-Widhopf et al., "Methods for the generation of chicken monoclonal antibody fragments by phage display", *J Immunol Methods* 242:159 (2000), and by Burton, D. R., "Phage display", *Immunotechnology* 1:87 (1995), the disclosures of which are incorporated herein by reference.

Cells derived from the transgenic animals of the present invention, such as B cells or cell lines established from a transgenic animal immunized against an antigen, are also part of the present invention.

In a further aspect of the present invention, methods are provided for treating a disease in a primate, in particular, a human subject, by administering a purified humanized antibody composition, preferably, a humanized polyclonal antibody composition, desirable for treating such disease.

In another aspect of the present invention, purified monoclonal or polyclonal antibodies are admixed with an appropriate pharmaceutical carrier suitable for administration in primates especially humans, to provide pharmaceutical compositions. Pharmaceutically acceptable carriers which can be employed in the present pharmaceutical compositions can be any and all solvents, dispersion media, isotonic agents and the like. Except insofar as any conventional media, agent, diluent or carrier is detrimental to the recipient or to the therapeutic effectiveness of the antibodies contained therein, its use in the pharmaceutical compositions of the present invention is appropriate. The carrier can be liquid, semi-solid, e.g. pastes, or solid carriers. Examples of carriers include oils, water, saline solutions, alcohol, sugar, gel, lipids, liposomes, resins, porous matrices, binders, fillers, coatings, preservatives and the like, or combinations thereof.

The humanized polyclonal antibody compositions used for administration are generally characterized by containing a polyclonal antibody population, having immunoglobulin concentrations from 0.1 to 100 mg/ml, more usually from 1 to 10 mg/ml. The antibody composition may contain immunoglobulins of various isotypes. Alternatively, the antibody composition may contain antibodies of only one isotype, or a number of selected isotypes.

In most instances the antibody composition consists of unmodified immunoglobulins, i.e., humanized antibodies prepared from the animal without additional modification, e.g., by chemicals or enzymes. Alternatively, the immunoglobulin fraction may be subject to treatment such as enzymatic digestion (e.g. with pepsin, papain, plasmin, glycosidases, nucleases, etc.), heating, etc., and/or further fractionated.

The antibody compositions generally are administered into the vascular system, conveniently intravenously by injection or infusion via a catheter implanted into an appropriate vein. The antibody composition is administered at an appropriate rate, generally ranging from about 10 minutes to about 24 hours, more commonly from about 30 minutes to about 6 hours, in accordance with the rate at which the liquid can be accepted by the patient. Administration of the effective dosage may occur in a single infusion or in a series of infusions. Repeated infusions may be administered once a day, once a week once a month, or once every three months, depending on the half-life of the antibody preparation and the clinical indication. For applications on epithelial surfaces the antibody compositions are applied to the surface in need of treatment in an amount sufficient to provide the intended end result, and can be repeated as needed. In addition, antibodies can, for example, be administered as an intramuscular bolus injection, which may, but does not have to, be followed by continuous administration, e.g. by infusion.

The antibody compositions can be used to bind and neutralize antigenic entities in human body tissues that cause disease or that elicit undesired or abnormal immune responses. An "antigenic entity" is herein defined to encompass any soluble or cell-surface bound molecules including proteins, as well as cells or infectious disease-causing organisms or agents that are at least capable of binding to an antibody and preferably are also capable of stimulating an immune response.

Administration of an antibody composition against an infectious agent as a monotherapy or in combination with chemotherapy results in elimination of infectious particles. A single administration of antibodies decreases the number of infectious particles generally 10 to 100 fold, more commonly more than 1000-fold. Similarly, antibody therapy in patients with a malignant disease employed as a monotherapy or in combination with chemotherapy reduces the number of malignant cells generally 10 to 100 fold, or more than 1000-fold. Therapy may be repeated over an extended amount of time to assure the complete elimination of infectious particles, malignant cells, etc. In some instances, therapy with antibody preparations will be continued for extended periods of time in the absence of detectable amounts of infectious particles or undesirable cells. Similarly, the use of antibody therapy for the modulation of immune responses may consist of single or multiple administrations of therapeutic antibodies. Therapy may be continued for extended periods of time in the absence of any disease symptoms.

The subject treatment may be employed in conjunction with chemotherapy at dosages sufficient to inhibit infectious disease or malignancies. In autoimmune disease patients or transplant recipients, antibody therapy may be employed in conjunction with immunosuppressive therapy at dosages sufficient to inhibit immune reactions. The invention is further illustrated, but by no means limited, by the following examples.

EXAMPLE 1

Isolation and Sequencing of BAC Clones Containing Rabbit Immunoglobulin Loci

High molecular weight DNA was isolated from a2b5 male rabbits. The rabbits were euthanized, spleen and kidneys were removed and rinsed in ice-cold PBS. Fat and connecting tissues were removed and processed separately. The organs were cut into pieces and homogenized in a pre-cooled Dounce homogenizer. The supernatant was transferred into

cooled 50 ml falcon tubes, mixed with cold PBS and large tissue debris was allowed to sink to the bottom for 2 minutes. Cells in the supernatant were pelleted at 200 g for 10 min at 4° C., washed once with PBS, resuspended in 1 ml PBS and counted. Sets of 5×10^6 , 5×10^7 and 5×10^8 cells were embedded in agarose plugs using the CHEF Mammalian Genomic DNA Plug Kit (BIORAD) To optimize conditions for partial digestion with HindIII, plugs were cut into 5 equal pieces and digested with 1 to 10 units of HindIII for various times and temperatures. Best results were obtained with 2 units HindIII at 4° C. for 3 hrs or 37° C. for 25 min. Digested DNA was double size fractionated on a Pulse Field Gel Electrophoresis (PFGE) apparatus using the following parameters: 6 hr backwards, 15s switch times; 6 hr forwards, 15s switch times; 20 hr forwards, 90s switch times; 200V 14° C. The area of the gel with the desired size of partial digested DNA was cut and DNA was isolated using gelase. 11 ng of insert was ligated with 1 ng of HindIII digested pBELOBAC 11 and electroporated into DH10B cells. 1% of the resulting colonies was sized using NotI and revealed an average insert size of 124 kb. 1×10^5 clones were spotted on Nylon filters and screened by hybridization with specific probes.

Probes for screening were amplified by PCR using genomic DNA from rabbits, cloned into pBlueScript, and verified by sequencing. Primer pairs (SEQ ID NO: 193-208, Table 2) were designed according to published sequences. Several BACs representing rabbit heavy and light chain immunoglobulin loci were isolated and mapped (FIGS. 1 and 5). BACs 219D23 219D23 (GenBank Acc. No. AY386695), 225P18 (GenBank Acc. No. AY386697), 27N5 (GenBank Acc. No. AY386696), 38A2 (GenBank Acc. No. AY386694), 179L1 (GenBank Acc. No. AY495827), 215M22 (GenBank Acc. No. AY495826), 19 (GenBank Acc. No. AY495828) and Fosmid Fos15B (GenBank Acc. No. AY3866968) were sequenced. Shotgun libraries for sequencing were constructed in pCR-Blunt with an insert size of 1.5-2 kb. For sequence analysis the STADEN package (Roger Staden, Cambridge, UK) was used. The software modules pregap and gap4 were used for assembly and gap closure. For the quality clipping of sequences PHRED (Washington University) and the STADEN package was coupled.

EXAMPLE 2

Construction of a Humanized Rabbit Immunoglobulin Heavy Chain Locus

BAC and fosmid clones containing rabbit immunoglobulin heavy chain locus sequences were isolated from genomic DNA libraries using probes specific for the constant, variable, and joining gene segments or the 3' enhancer region. Isolated BACs (FIG. 1) 27N5 (GenBank Acc. No. AY386696), 219D23 (GenBank Acc. No. AY386695), 225P18 (GenBank Acc. No. AY386697), 38A2 (GenBank Acc. No. AY386694) and fosmid Fos15B (GenBank Acc. No. AY3866968) were sequenced (Ros et al., *Gene* 330, 49-59).

Selected immunoglobulin coding sequences were exchanged with corresponding human counterparts by homologous recombination in *E. Coli* by ET cloning (E-Chiang Lee et al., *Genomics* 73, 56-65 (2001); Daiguan Yu et al., *PNAS* 97, 5978-5983 (2000); Muyrers et al., *Nucleic Acids Research* 27, 1555-1557 (1999); Zhang et al., *Nature Biotechnology* 18, 1314-1317 (2000)).

Alternatively, DNA fragments were recombined by ligation in vitro and subsequent transformation of *E. coli*. BACs

and/or Fos15B or parts thereof were combined by in vitro ligation and transformation, ET cloning, or by Cre recombinase mediated integration.

For ET cloning, vectors containing target sequence were transformed into a streptomycin resistant *E. coli* strain containing the inducible lambda phage recombination enzymes Red α , Red β and γ . These recombination proteins were expressed either from a co-transfected plasmid (DH10B *E. coli* cells with plasmid pSC101) or from a genomic integrated lambda prophage (DY380 *E. coli* strain). The ET cloning procedure encompassed two homologous recombination steps.

In a first step the target locus was replaced by a selection-counter selection cassette (e.g. neo-rpsL which confers resistance to neomycin (neo) and sensitivity to streptomycin (rpsL)). After isolation of neo-resistant colonies, insertion of the selection cassette by homologous recombination was confirmed by restriction enzyme analysis and partial sequencing.

In a second step, the rpsL-neo selection cassette was exchanged with a new sequence. Streptomycin resistant clones were analyzed by restriction analysis and sequencing. Fragments used for the ET cloning procedure had flanking sequences of 20 to 50 bp length, which were identical to target sequences. Sequences used for ligation had appropriate restriction enzyme sites at their 3' and 5' ends. These sites were either naturally occurring sites or they were introduced by PCR using primers containing appropriate sites.

Alternatively, sequences were generated synthetically.

A humanized heavy chain was constructed by replacement of rabbit J_H, C μ in BAC 219D23 and C γ in BAC 27N5 with their corresponding human counterparts by ET cloning. Human sequences used for the ET cloning procedures were amplified by PCR from human genomic DNA.

Human C μ , C γ and J_H gene segments was amplified using primers (SEQ ID Nos: 209-214, Table 2) with 50 bp homologies to rabbit target sequences.

After ligation of BAC clone 225P18 with clone 219D23 and BAC 27N5 with Fosmid 15B, the ligated constructs were transformation into *E. coli* and connected by Cre recombinase mediated insertion. This resulted in a functional locus consisting of 18 rabbit variable genes, rabbit D region, human J region, human C μ , human C γ , rabbit C ϵ , rabbit C α 4 and the 3' enhancer element.

For the generation of transgenic animals the humanized BAC clones were coinjected either separately as three overlapping BACs (225P18 and 219D23 and BAC 27N5) or two overlapping combined BACs (225P18-219D23 and BAC 27N5-Fosmid 15B) or as one BAC (225P18-219D23-27N5-Fosmid15B). Founder animals with transgenes were identified by PCR.

EXAMPLE 3

Construction of a Humanized Immunoglobulin Heavy Chain Locus Using Synthetic Fragments

Four fragments denoted Unit1, Unit2, Unit3, and Unit 4 (FIG. 13, SEQ ID Nos: 189-192) with human V sequences and rabbit spacers were chemically synthesized. Each fragment was flanked 5' by an AscI restriction endonuclease recognition sequence, 3' by a lox71 Cre recombinase recognition sequence followed by Fse I and MluI restriction enzyme recognition sequences. Unit 1 consisted of human V_H3-49, V_H3-11, V_H3-7 and V_H3-15 variable genes separated by rabbit spacers I29-30, I3-4, I2-3 and the 3' half of I1-2 (I1-2B). Unit 2 consisted of human V_H3-48, V_H3-43 and V_H3-64 separated by rabbit spacers I1-2A (5' half of I1-2), I7-8, I6-7

and the 3' half of I4-5 (I4-5B). Unit 3 consisted of human V_H3-74, V_H3-30, and V_H3-9 separated by the rabbit spacer sequences I4-5B, I26-27, I11-12 and I17-18.

Unit 3 had in addition to the afore mentioned upstream flanks an Flp recombinase recognition target (FRT) sequence, followed by a Sglf I restriction endonuclease recognition sequence preceding the already mentioned Asc I site.

Unit 4 had the human V_H3-23 gene 5' flanked by the rabbit spacer I1-2, a lox66 Cre recombinase target sequence and an AscI endonuclease recognition sequence, and 3' flanked by IV-C (5' half) rabbit spacer sequence followed by a MluI endonuclease recognition sequence.

A gentamycin selection cassette was PCR-amplified, using primers SEQ ID NOs 215 and 216 (Table 2) containing AscI and FseI sites and ligated into a pGEM vector with a modified cloning site including AscI, FseI, and MluI endonuclease recognition sites (pGEM.Genta modified by PCR using SEQ ID NOs 217 and 218, Table 2).

Units 1, 2 and 3 were cloned into pGEM.Genta (Promega) vectors.

Unit 4 was sub-cloned into a customized pBELOBAC11 (NEB) vector linearized with Hind III, and PCR-amplified. The forward primer (SEQ ID NO: 219, Table 2) had restriction sites for HindIII, PacI and AatII, and the reverse primer (SEQ ID NO: 220) sites for Bam HI, MluI and AscI The primers were designed in such a way that the pBELOBAC11 Chloramphenicol selection cassette was deleted. Furthermore, a Neomycin selection cassette was PCR-amplified with primers SEQ ID NOs 221 and 222 (Table 2) carrying Bam HI and Hind III restriction sites and ligated to the modified pBELOBAC11 vector (pBB11.1).

Units 1-4 were assembled by cre-mediated recombination as described (Mejia et al, *Genomics* 70(2) 165-70 (2000)). First Unit 1 was cloned into the customized pgem.Genta vector, digested with Fse I and subsequently recircularized by ligation. This vectorless construct was transformed into *E. coli* containing pBB11.1. Unit4 and p706-Cre plasmid. Following recombination of Unit 1 with PBB11.1 unit 4, positive clones (Unit4/1) were selected on kanamycin and gentamycin containing media. Clones were characterized by restriction analyses using various enzymes.

For recombination of Unit 2, the Unit4/1 insert was excised by double digestion with AscI and PacI, and cloned into pBELOBAC11 with a modified linker (pBB11.2: modified by PCR using primers SEQ ID NOs 223 and 224, Table 2).

pBELOBAC11 was linearized with HindIII and PCR-amplified with a forward primer encoding PacI and AatII endonuclease recognition sites and a reverse primer encoding MluI and NotI endonuclease recognition sites and a lox66 Cre recombinase target site. For ligation with Unit1/4 the pBB11.2 vector was opened with MluI and PacI. pGEM.Genta.Unit2 was converted into a circular vectorless construct as described for pGEM.Genta.Unit1 and connected with pBB11.2 Unit4/1 by in vivo Cre mediated recombination. Subsequently, the resulting construct pBB11.2 Unit4/1/2 is prepared for Cre mediated recombination with Unit 3 by replacing the wild type loxp site with a lox66 target site by ET-cloning (Muyers et al., *Nucleic Acids Research* 27, 1555-1557 (1999); Muyers et al *Trends Biochem. Sci.* 26(5):325-31 (2001)). A chloramphenicol selection cassette was amplified by PCR with primers (SEQ ID NOs 225 and 226, Table 2) containing 50 bp sequences homologous to the BAC target sequence. The reverse primer included a lox66 site. The gel-purified PCR product was transformed into cells carrying the target BAC as well as the pSC101 plasmid, required for homologous recombination. Positive clones were selected with chloramphenicol and confirmed by restriction analysis

and sequencing. pGEM.Genta.Unit 3 was prepared for in vivo recombination as described above for Unit 1 and 2 and transformed into cells carrying the receptor BAC, as well as the p706-Cre plasmid. Positive clones pBB11.2 Unit4/1/2/3 were selected with gentamycin and confirmed by restriction analysis. pBB11.2 Unit4/1/2/3 was further modified by ET-cloning to generate a lox 71 target site. Subsequently, pBB11.2 Unit4/1/2/3 was connected to fragments from BACs 219D23, 27N5 and Fos15B.

EXAMPLE 4

Construction of a Humanized Immunoglobulin Heavy Chain Locus Using PCR Amplified Fragments

Human V_H elements were amplified using genomic DNA (ClonTech) and primers SEQ ID NOs 227-248 (Table 2). PCR products were analyzed by gel-electrophoresis and gel purified using the GENECLAN kit (Q-Biogen). Subsequently, amplification products were sub-cloned into Zero-Blunt TOPO™ (Invitrogen), according to the manufacturer's instructions. The sequences of all amplified V elements were confirmed. For the construction of the humanized V region, V elements were amplified using plasmid DNA as template and primers SEQ ID NOs 249-270 (Table 2). Forward primers contained an AscI site, followed by a rabbit splice site. Reverse primers contained a rabbit recombination signal sequence (RSS) and a MluI restriction site. PCR products were gel purified using the GENECLAN kit.

Human $V_{H,S}$, V3-33, V3-74, V3-49, V3-21, V3-48, V3-73, V3-7, and V3-D could not be isolated by PCR and were synthesized chemically (BlueHeron, Bothel, Wash.). Restriction sites and rabbit regulatory sequences were added during synthesis.

Rabbit spacer sequences were amplified using BACs 38A2 and 225P18 as templates and primers SEQ ID NOs 271-288 (Table 2). BAC 225P18 was double digested with NheI and a 41 kb fragment was gel purified. This fragment served as a template for the amplification of spacers V1-2, V2-3, V3-4, V4-5, and V5-6.

BAC 225P18 was digested with BstBI and the template for spacers V6-7 and V7-8 was gel-purified. A double digestion of BAC 38A2 with PacI and RsrII allowed gel purification of the template for spacers V21-22, and V22-23.

Amplified spacer sequences were gel-purified, and sub-cloned into XL-PCR-TOPO™ (Invitrogen) according to the manufacturer's instructions.

V_H elements and rabbit spacer sequences were sub-cloned into modified pGEM (Promega) and pBS (Stratagene) vectors. The pGEM vector was cut with NotI and Hind III and ligated with chemically synthesized oligonucleotide sequences containing FseI, AscI and MluI sites (Oligo1: SEQ ID NO: 289; Oligo2: SEQ ID NO: 290; Table 2). Vector pBS was cut with SacI and KpnI and ligated with a chemically synthesized oligonucleotide sequence containing the restriction sites FseI, AscI and MluI (Oligo1: (SEQ ID NO: 291; Oligo2: SEQ ID NO: 292, Table 2).

Gentamycin and neomycin selection cassettes were amplified using primers (SEQ ID NOs: 293-296, Table 2) with FseI or AscI sites and ligated into the modified pGEM and pBS-vectors.

The final construct is built in a modified pBeloBAC II vector. The pBeloBAC II vector was opened with BamHI and HindIII and the cloning sites were modified to contain FseI,

AscI, MluI sites using a chemically synthesized oligonucleotide sequence (Oligo1: SEQ ID NO: 297; Oligo2: SEQ ID NO: 298, Table 2).

BAC 219D23 was modified by introduction of restriction sites using ET-cloning (Muyers et al., *Nucleic Acids Research* 27, 1555-1557 (1999); Muyers et al *Trends Biochem. Sci.* 26(5):325-31 (2001)). The Neomycin selection cassette was amplified with primers SEQ ID NO: 299 and SEQ ID NO: 300 (Table 2). The forward primer contained an FseI site, the reverse primer an AscI site.

The purified PCR product was transformed into *E. coli* cells carrying BAC 219D23 and plasmid pSC101 necessary for homologous recombination. After homologous recombination of the cassette and the target sites in the BAC, introduced restriction sites were confirmed by restriction analysis. Subsequently, the modified BAC 219D23 was digested with FseI and MluI and the resulting 17 kb fragment (containing the FseI-Neo-AscI cassette) was separated by PFGE and purified by electro-elution. This purified fragment was ligated with the modified pBeloBAC II vector opened with FseI and MluI.

A purified DNA fragment encoding a human V_H element is ligated with the modified pGEM.neo vector opened with AscI and MluI. Similarly a spacer sequence is sub-cloned into the modified pGEM.genta vector. Subsequently, the pGEM.genta vector carrying the spacer sequence is cut with FseI and MluI and the insert is ligated with pGEM.neo. V_H vector opened with FseI and AscI. This step is repeated several times to build a fragment consisting of several spacer and V_H segments. Such fragments are excised with FseI and MluI and ligated with the modified pBeloBAC II vector linearized with FseI and AscI. These processes are repeated to build a large immunoglobulin V region (FIG. 6). The humanized heavy chain locus is used for the generation of transgenic animals.

EXAMPLE 5

Construction of a Humanized Rabbit Light Chain Locus Containing Humanized Ck and Humanized Rearranged VJ

Screening of a rabbit genomic BAC libraries resulted in the identification of three BACs (215M22, 179L1 and 196O2; Gene Bank Accession Nos: AY495826, AY495827, and AY495828, respectively) containing rabbit light chain K1 gene segments. Rabbit Ck1 was exchanged with human Ck allotype Km3 by ET cloning as described (E-Chiang Lee et al., *Genomics* 73, 56-65 (2001); Daiguan Yu et al., *PNAS* 97, 5978-5983 (2000); Muyers et al., *Nucleic Acids Research* 27, 1555-1557 (1999); Zhang et al., *Nature Biotechnology* 18, 1314-1317 (2000)). Human Ck (allotype Km3) was amplified by PCR with primers (SEQ ID Nos 301 and 302, Table 2) containing 50 bp sequences homologous to target sequences. Homology arms were designed based on the published sequence of rabbit germline kappa (b5; GenBank Accession No. K01363) and matched the intron-exon boundary of Ck. The exchange of rabbit Ck against the human Ck in BAC 179L1 was verified by sequencing.

BAC 179L1-huCk was modified by two ET cloning. A neomycin selection cassette was amplified with primers (SEQ ID NOs 303 and 304, Table 2) containing 50 bp sequences homologous to BAC 179L1. The forward primer additionally had an i-CeuI meganuclease site. The PCR product was used for ET cloning. Positive clones were selected with neomycin and checked for correctness by restriction enzyme digests and sequencing. A zeocin selection cassette was amplified with primers (SEQ ID NOs 305 and 306, Table

2) containing 50 bp sequences homologous to BAC 179L1. The forward primer additionally had an i-SceI meganuclease site. The PCR product was used for ET cloning. Positive clones were selected with zeozin and checked for correctness by restriction enzyme digests and sequencing.

BAC 215M22 was modified by one ET cloning. A gentamycin resistance gene was amplified with primers (SEQ ID NOs 307 and 308, Table 2) containing 50 bp sequences homologous to BAC215M22. The forward primer additionally had an i-CeuI Meganuclease site and the reverse primer an i-SceI meganuclease site. The PCR product was used for ET cloning. Resulting clones were selected with gentamycin and analyzed by restriction enzyme digests and sequencing.

Modified BAC179L1 and 225M22 were cut with i-CeuI and i-SceI. Fragments of 98 kb and 132 kb were purified and ligated. Resulting clones were selected with kanamycin and chloramphenicol and checked for correctness by restriction enzyme digests, PCR of the regions containing i-SceI and i-CeuI restriction sites, and sequencing. The resulting BAC was termed 179-215-huCk.

Rabbit J κ 1 and J κ 2 of BAC 179-215-huCk were replaced by ET cloning with a synthetic human rearranged V κ J κ gene. A DNA fragment with rabbit promoter, rabbit leader, rabbit intron and human V κ J κ gene was synthesized chemically. The codon usage of the synthetic human VJ was optimised to achieve highest DNA sequence homology to rabbit V kappa genes.

The synthetic human VJ was PCR amplified with a forward primer (SEQ ID NO 309, Table 2) containing 50 bp sequences homologous to BAC 179L1 and a reverse primer (SEQ ID NO 310, Table 2) containing a sequence homologous to the gentamycin resistance gene and a FRT site. A gentamycin resistance gene was amplified with a forward primer (SEQ ID NO 311, Table 2) containing a FRT site and a reverse primer (SEQ ID NO 312, Table 2) with 50 bp homology to BAC 179L1 and a FRT site. The human synthetic human VJ and the gentamycin resistance gene were combined by overlap extension PCR using the forward primer for the synthetic human VJ gene and the reverse primer for the gentamycin resistance gene. The resulting fragment was used for ET cloning. Positive clones were selected with gentamycin and checked for correctness by restriction enzyme digests and sequencing.

The gentamycin resistance gene was removed by site specific recombination via expression of Flp recombinase. After recombination one FRT was left. The FRT site was deleted by ET cloning. A 232 bp fragment from the synthetic human VJ was amplified by PCR (using primers SEQ ID NOs 313 and 314, Table 2) and used for ET cloning. Resulting colonies were screened by PCR (using primers SEQ ID NOs 315 and 316, Table 2) for loss of the FRT site and confirmed by sequencing.

The neomycin resistance gene of BAC179-215-huCk was replaced by ET cloning. A gentamycin resistance (pRep-Genta; Genebridges) gene was amplified by PCR with primers (SEQ ID NOs 317 and 318, Table 2) containing 50 bp sequences homologous to BAC 179-215-huCk. The forward primer additionally had a loxP site, an attB site and a PvuI restriction site. Resulting clones were selected with gentamycin and checked for correctness by restriction enzyme digests and sequencing.

The resulting BAC (rLC3-B; FIG. 8) was used for the generation of transgenic animals.

Construction of a Humanized Rabbit Light Chain Locus Containing Multiple Human V κ Elements, Chicken Spacer Elements and a Rearranged Human VJ

Screening of a rabbit genomic BAC libraries resulted in the identification of three BACs (215M22, 179L1 and 196O2; Gene Bank Accession Nos: AY495826, AY495827, and AY495828, respectively) containing rabbit light chain K1 gene segments. Rabbit C κ 1 was exchanged with human C κ allotype Km3 by ET cloning as described (E-Chiang Lee et al., *Genomics* 73, 56-65 (2001); Daiguan Yu et al., *PNAS* 97, 5978-5983 (2000); Muyrers et al., *Nucleic Acids Research* 27, 1555-1557 (1999); Zhang et al., *Nature Biotechnology* 18, 1314-1317 (2000)). Human C κ (allotype Km3) was amplified by PCR with primers (SEQ ID Nos 301 and 302, Table 2) containing 50 bp sequences homologous to target sequences. Homology arms were designed based on the published sequence of rabbit germline kappa (b5; GenBank Accession No. K01363) and matched the intron-exon boundary of C κ . The exchange of rabbit C κ (against the human C κ in BAC 179L1) was verified by sequencing.

Two DNA fragments, Unit1 (12,235 bp, FIG. 12a, SEQ ID NO 187,) containing 17 human V pseudogenes and 18 chicken spacer sequences and Unit 2 (13,283 bp, FIG. 12b, SEQ ID NO 188) containing one functional rearranged human kappa VJ gene with leader, 11 human V pseudogenes, 12 chicken spacer sequences and intron1 and parts of intron 2 were synthesized chemically and cloned into vector pBR322.

Units 1 and 2 were digested with the restriction enzyme NgoMIV and AsiSI or NgoMIV and AscI respectively and ligated into pBELOBAC11 with a modified linker by three fragment ligation. The modified linker contained a BsiWI restriction site, a FRT5-site, a rpsL-Neo-cassette, a AscI site and a AsiSI-site. The linker fragment was amplified with High fidelity polymerase (Roche), primers CE_1_001_012904 (SEQ ID NO 319, Table 2) and CE_1_on005_013004 (SEQ ID NO 320, Table 2) and plasmid pRpsL-Neo (Genebridges) as template. Subsequently, the amplified product was ligated into BamHI and HindIII sites of pBELOBAC11. For ligation with Unit 1 and 2 the modified pBELOBAC11 was opened with AsiSI and AscI. Positive clones (pBELOBAC11 Unit1/2) were checked by restriction enzyme digests.

BAC 179L1 (GENBANK Acc. No. AY495827) was modified by insertion of two modified selection cassettes by ET cloning. Cassette 1 was a gentamycin resistance gene amplified with primers (SEQ ID Nos 321 and 322, Table 2) containing 50 bp sequences homologous to BAC 179L1 and an AscI site in the reverse primer. Cassette 2 was a rpsL-Neo selection cassette amplified with primers (SEQ ID Nos 323 and 324, Table 2) containing 50 bp sequences homologous to BAC 179L1 and an attB site, a FRT5 site and a BsiWI site in the forward primer.

The purified PCR products were transformed into *E. coli* cells carrying the BAC and plasmid pSC101 necessary for homologous recombination. After homologous recombination successful modification of the BAC was confirmed by restriction digest analyses, Southern Blot and sequencing.

Modified BAC 179L1 was cut with the restriction enzymes AscI and BsiWI. The fragment containing the human C κ (was purified and ligated with pBELOBAC11 Unit1/2 opened with the same restriction enzymes. Positive clones were checked

by restriction enzyme digests. The final construct (FIG. 12c) is used for the generation of transgenic animals

EXAMPLE 7

Construction of a Humanized Rabbit Light Chain Locus Containing Multiple Human V κ Elements, Chicken Spacers and an Unrearranged Human J Kappa Locus

The construct described in example 6 was modified by ET cloning as follows: an. The rearranged functional VJ sequence was exchanged with a functional V1 flanked by a functional recombination signal sequence (RSS). The RSS was PCR amplified from BAC179L1 with a forward primer (SEQ ID NO 325, Table 2) containing a 50 bp sequence homologous to V1 of pBELOBAC11 Unit1/2 and a reverse primer (SEQ ID NO 326, Table 2) containing an AscI restriction enzyme site and homology to the gentamycin resistance gene. A gentamycin resistance gene was amplified with a forward primer (SEQ ID NO 327, Table 2) containing a sequence homologous to the reverse primer used for RSS amplification and a reverse primer (SEQ ID NO 328, Table 2) containing a 50 bp sequence homologous to pBELOBAC11 Unit1/2 and a BsiWI restriction enzyme site.

The RSS and the gentamycin resistance gene were combined by overlap extension PCR using the forward primer for RSS amplification and the reverse primer for Gentamycin resistance gene amplification. The resulting fragment was used to modify pBELOBAC11 Unit1/2 by ET cloning. Positive clones were selected with gentamycin and analyzed by restriction enzyme digests and sequencing.

BAC 179L1 with human C κ was further modified by ET cloning. A kanamycin selection cassette was amplified with a primers (SEQ ID NO 329 and 330, Table 2) containing 50 bp sequences homologous to BAC 179L1. The reverse primer contained also an AscI restriction enzyme site and a FRT site. The PCR product was used for ET cloning. An ampicillin selection cassette was amplified with primers (SEQ ID Nos 331 and 332, Table 2) containing 50 bp sequences homologous to BAC 179L1. The forward primer contained also an attB site, an AsiSI restriction enzyme site and a FRT5 site. The reverse primer contained a BsiWI restriction enzyme site and a FRT site. The PCR product was used for ET cloning. The human J region was amplified from human genomic DNA with primers (SEQ ID Nos 333 and 334, Table 2) containing 50 bp sequences homologous to BAC 179L1. The PCR product was used for ET cloning. The resulting clones were analyzed by restriction enzyme digest and sequencing.

A positive clone was cut with AscI and BsiWI. The resulting fragment was purified and ligated into the modified pBELOBAC11 Unit1/2 cut with the same enzymes. Positive clones were selected with ampicillin and analyzed by restriction enzyme digests and sequencing. Correct clones are used to generate transgenic animals.

EXAMPLE 8

Construction of a Humanized Rabbit Light Chain Locus Containing Multiple Human V κ Elements

Screening of a rabbit genomic BAC libraries resulted in the identification of three BACs (215M22, 179L1 and 196O2; Gene Bank Accession Nos: AY495826, AY495827, and AY495828, respectively) containing rabbit light chain K1 gene segments. Rabbit C κ 1 was exchanged with human C κ allotype Km3 by ET cloning as described (E-Chiang Lee et

al., *Genomics* 73, 56-65 (2001); Daiguan Yu et al., *PNAS* 97, 5978-5983 (2000); Muyrers et al., *Nucleic Acids Research* 27, 1555-1557 (1999); Zhang et al., *Nature Biotechnology* 18, 1314-1317 (2000)). Human C κ (allotype Km3) was amplified by PCR with primers (SEQ ID NOs 301 and 302, Table 2) containing 50 bp sequences homologous to target sequences. Homology arms were designed based on the published sequence of rabbit germline kappa (b5; GenBank Accession No. K01363) and matched the intron-exon boundary of C κ . The exchange of rabbit C κ against the human C κ in BAC 179L1 was verified by sequencing.

Human V κ elements of the V κ 1 family (O2, L8, L4, A30, L11, L1, L5, L15, O8, L19, L12, A20, O4, L14, L23, L9, A4, L24, O6, L22, A9, A25, A15, O9) were amplified by PCR using primers (SEQ ID NOs 335-382, Table 2) and human genomic DNA as a template.

Amplification products were analysed by gel-electrophoresis, gel purified using GENECLEAN (Q-Biogen), subcloned into the Zero-Blunt TOPO™ vectors (Invitrogen) and sequenced. A rearranged human V κ (O2) J κ (J4) element was produced by PCR amplification, subcloned and sequenced. To combine human V κ elements with rabbit spacers, human V κ elements were amplified by PCR with primers (SEQ ID Nos 383-430, Table 2) using plasmid DNA as a template. Primers contained AscI or MluI sites.

Rabbit spacer sequences are amplified by PCR using primers SEQ ID NOs 431-450 (Table 2). BACs 179L1 and 215M22 are digested with SpeI, NheI, AclI, SfoI, MluI, and Sall/XhoI. Fragments are gel purified and used as amplification templates.

The spacer sequence located at the 5-end is amplified by an upstream oligonucleotide containing a FRT and an attB site. PCR products are gel purified using the GENECLEAN kit and subcloned into XL-PCR-TOPOTM (Invitrogen) according to the manufacturer's instructions.

Human V κ elements and rabbit spacer sequences were cloned into pGem (Promega) modified as described in Example 4.

Human V kappa and the modified pGEM.genta vector are digested with AscI and MluI and ligated. Similarly, rabbit spacer sequences are cloned into pGEM.neo. Subsequently, pGem.neo.V κ is cut with FseI and AscI and ligated with a purified insert of pGem.genta.spacer excised with FseI and MluI. Ligation of AscI and MluI complementary ends destroys the restriction enzyme site and allows repeated use of AscI and MluI for the construction of a V κ locus comprising several V κ and spacer elements. The final construct, consisting of fragments of a humanized BAC 179L1 and 215M22 and a humanized V κ region is built in pBeloBAC. BAC 179L1 and 215M22 were modified and combined. Subsequently, BAC 179L1-215M22-huC κ was further modified by ET cloning. Two cassettes containing restriction enzyme site, selection markers, and additional functional sites were inserted into the vector by ET-cloning as shown in FIG. 7. Primers (SEQ ID NOs 451-454) used for the amplification of the cassettes are listed in Table 2.

To built the final construct, units consisting of human V elements, rabbit spacer elements and a resistance marker are excised out of pGEM with FseI and MluI and ligated with BAC 179L1-215M22 digested with FseI and AscI. Subsequently, the resistance marker is replaced with a new insert consisting of human V elements, rabbit spacer elements and another resistance marker. After several repeats the final construct will consist of many V κ segments (L8, L4, A30, L11, L1, L5, L15, O8, L19, L12, A20, O4, L14, L23, L9, A4, L24, O6, L22, A9, A25, A15, O9) separated by rabbit spacer

sequences. The humanized light chain locus is used for the generation of transgenic animals.

EXAMPLE 9

Construction of a Humanized Heavy Chain Locus with Chicken Heavy Chain Locus Spacer Sequences

A synthetic humanized heavy chain locus containing a rabbit D region, a human J region, human C μ , human C γ , rabbit C α 4, the rabbit 3' α enhancer and human VH elements (including promotor and nonamer/heptamer sequences) separated by chicken spacer sequences is constructed.

Modified rabbit BAC 27N5 (see "Example 2) was further modified by ET cloning. The construct contained a humanized C μ and C γ and two unique restriction sites, BsiWI and AsiSI downstream of the α -4 membrane exon. DNA is amplified with oligonucleotides SEQ ID Nos 455 and 456 (Table 2).

Fosmid Fos15B is digested with NheI and the resulting 13 kb fragment containing the 3' α enhancer is subcloned into a cloning vector in such a way that the insert is flanked by BsiWI and AsiSI sites. Subsequently, the insert is excised with BsiWI and AsiSI and ligated with the modified BAC 27N5 to form BAC 27N5Fos.

The rabbit J region in BAC219D23 was exchanged with the corresponding human J region by ET cloning. The human J region was amplified by PCR using primers SEQ ID NOS 457 and 458 (Table 2).

Unique restriction enzyme sites are inserted in BAC219D23 upstream of the D region (A) and upstream of C μ (B). In BAC 27N5Fos restriction site A is inserted upstream of the linker region and B is inserted in sequences homologous to BAC219D23. Following digestion with enzymes A and B, the fragment containing human J and rabbit D regions is isolated and ligated with BAC 27N5Fos to create BAC 219D23/27N5Fos.

Chicken heavy chain spacer sequences are amplified from chicken genomic DNA by PCR using primers (SEQ ID NOS 459 and 460, Table 2) specific for chicken heavy chain V pseudogenes (Mansikka et al., *J Immunol* 145(11), 3601-3609 (1990), Reynaud et al., *Cell* 59(1), 171-183 (1989)). Alternatively, spacer sequences are synthesized chemically.

The PCR products are gel purified, cloned into pTOPO (Invitrogen) and sequenced.

Human heavy chain variable elements are amplified by PCR using primers designed according to published sequences in GENBANK (eg Acc. No. NG_001019) or synthesized chemically. The human V elements contain the human promoter region, the human leader sequence, the human intron between leader and V-coding region, the human V-coding region and the human recombination signal sequence. The amplified or synthesized fragments are flanked by specific restriction endonuclease recognition. Chicken spacer sequences and human V elements are combined in one or several large DNA fragment comprising a humanized immunoglobulin locus. The construct is used to generate transgenic animals.

EXAMPLE 10

Construction of a Humanized Immunoglobulin Locus Containing Human V Elements and Non-Human Spacer Sequences (Without Promotor Region and RSS)

A BAC library generated with non-human genomic DNA is screened with probes specific for immunoglobulin and

BAC clones containing heavy and light chain immunoglobulin C, J and D regions are identified. The BAC clones are modified to contain restriction enzyme sites. Human heavy and light chain variable elements are amplified by PCR using primers designed according to published sequences in GenBank (eg., Acc. No. NG_001019). Sequences are amplified from genomic DNA or synthesized chemically. The human V elements contain the human promoter region, the human leader sequence, the human intron between leader and V-coding region, the human V-coding region and the human recombination signal sequence (RSS). The amplified or synthesized fragments have specific restriction endonuclease recognition sites at the ends. The non-human spacer sequences are amplified by PCR or synthesized chemically. Non-human spacer sequences and human V elements are combined in one or several large DNA fragment comprising a humanized immunoglobulin locus. The construct is used to generate transgenic animals. An example for the construction of a humanized V region using chicken spacer sequences is shown in FIG. 10.

EXAMPLE 11

Construction of a Humanized Immunoglobulin Locus Containing Human V Elements and Non-Human Spacer Sequences

A BAC library generated with non-human genomic DNA is screened with probes specific for immunoglobulin and BAC clones containing heavy and light chain immunoglobulin C, J and D regions are identified. The BAC clones are modified to contain restriction enzyme sites. Human heavy and light chain variable elements are amplified by PCR using primers designed according to published sequences in GenBank (eg., Acc No. NG_001019). Sequences are amplified from genomic DNA or synthesized chemically. The human V elements contain the human V coding region. Non-human spacer sequences are amplified by PCR or synthesized chemically and contain a recombination signal sequence, a spacer sequence, a promotor region, a leader sequence and the intron between leader and V coding region. Such non-human spacer sequences are combined with human V elements in one or several large DNA fragments and used for the generation of transgenic animals. An example for the construction of a humanized V region using mouse or rabbit spacer sequences is shown in FIG. 11.

EXAMPLE 12

Transgenic Rabbits Expressing Humanized Immunoglobulins

Transgenic rabbits were generated as described by Fan et al. (*Pathol. Int.* 49: 583-594, 1999). Briefly, female rabbits are superovulated using standard methods and mated with male rabbits. Pronuclear-stage zygotes are collected from oviduct and placed in an appropriate medium such as Dulbecco's phosphate buffered saline supplemented with 20% fetal bovine serum. BAC containing humanized immunoglobulin loci were microinjected into the male pronucleus with the aid of a pair of manipulators. Morphological surviving zygotes were transferred to the oviducts of pseudopregnant rabbits. Pseudopregnancy was induced by the injection of human chorionic gonadotrophin (hCG). Following injection of a humanized light chain construct into 4645 pronuclei of fertilized oocytes, 4043 oocytes were transferred into 132 recipients. In total, 253 live offspring were born, 11 of which were transgenic. Expression of human kappa light chain was

detected by ELISA using human-kappa light chain specific reagents (for example, mouse anti-human Kappa, Southern Biotech, 9220-01; goat anti-human Kappa, Southern Biotech 2063-08).

A humanized heavy chain construct was injected into 4083 pronuclei of fertilized oocytes. 3485 oocytes were transferred into 119 recipients which delivered 433 offspring. Analysis by PCR and FISH revealed that 20 of these animals were transgenic. Humanized heavy chain in the blood of founder animals was detected by ELISA using antibodies specific for human IgM/IgG (for example, rabbit anti-human IgM, Rockland 609-4131; rabbit anti-human IgM, Rockland 609-4631; rabbit anti-human IgG, Pierce 31142, rabbit anti-human IgG, Southern Biotech 6145-08; rabbit anti-human IgG, Pierce 31784).

Sandwich-type ELISAs detecting humanized κ , μ and γ chains were performed using standard procedures. Briefly, microtiter plates were coated with capture antibody and incubated with diluted serum samples. Bound human immunoglobulin was detected using a secondary labeled antibody and peroxidase-streptavidin-conjugate (Sigma, S2438).

Double transgenic animals expressing both humanized heavy and light immunoglobulin chains were generated by breeding of founder animals.

EXAMPLE 13

Transgenic Mice Expressing Humanized Immunoglobulins

Transgenic mice were generated as described by Nagy et al. (*Manipulating the Mouse Embryo: A Laboratory Manual; Cold Spring Harbor Laboratory Press, New York, 2003*).

Briefly, female mice were superovulated using standard methods and mated with male mice. Pronuclear-stage zygotes were collected from oviduct and placed in a suitable medium such as M2 medium. BAC containing humanized immunoglobulin loci were microinjected into the male pronucleus with the aid of a pair of manipulators. Morphologically surviving zygotes were transferred to the oviducts of pseudopregnant female mice. Pseudopregnancy was induced by mating with sterile males. Following injection of a humanized light chain construct into 1325 pronuclei of fertilized oocytes, 787 oocytes were transferred into 29 recipients. In total, 55 live offspring were born, 11 of which were transgenic.

A humanized heavy chain construct was injected into 1050 pronuclei of fertilized oocytes. 650 oocytes were transferred into 25 recipients which delivered 64 live offspring. Analysis by PCR revealed that 19 of these animals were transgenic.

Double transgenic animals expressing both humanized heavy and light immunoglobulin chains are generated by breeding of founder animals. Expression of humanized κ , μ and γ chains was detected by ELISAs using standard procedures. Briefly, microtiter plates were coated with capture antibody and incubated with diluted serum samples. Bound human immunoglobulin was detected using a secondary labeled antibody and peroxidase-streptavidin-conjugate (Sigma, S2438).

All references cited throughout the specification are hereby expressly incorporated by reference. While the invention is illustrated by reference to certain embodiments, it is not so limited. One skilled in the art will recognize that various modifications and variations are possible without diverting from the essence of the invention. All such modifications and variations are specifically included within the scope herein.

TABLE 1

ID	BAC	Accession#	Start	Finish	Description
1	38A2	AY386694	1	2137	Spacer 5' start-V34
2	38A2	AY386694	2433	9504	Spacer V34-V33
3	38A2	AY386694	9798	19384	Spacer V33-V32
4	38A2	AY386694	19690	35164	Spacer V32-V31
5	38A2	AY386694	35447	47669	Spacer V31-V30
6	38A2	AY386694	47973	52521	Spacer V30-V29
7	38A2	AY386694	52819	61798	Spacer V29-V28
8	38A2	AY386694	62100	74264	Spacer V28-V27
9	38A2	AY386694	74566	79145	Spacer V27-V26
10	38A2	AY386694	79449	84800	Spacer V26-V25
11	38A2	AY386694	85103	95717	Spacer V25-V24
12	38A2	AY386694	96009	102226	Spacer V24-V26
13	38A2	AY386694	102504	105307	Spacer V23-V22
14	38A2	AY386694	105603	107583	Spacer V22-V24
15	38A2	AY386694	107769	118033	Spacer V24-V20
16	38A2	AY386694	118334	125546	Spacer V20-V19
17	38A2	AY386694	125849	128059	Spacer V19-3' end
18	225P18	AY386697	1	4333	Spacer 5' start-V18
19	225P18	AY386697	4629	9255	Spacer V18-V17
20	225P18	AY386697	9561	15841	Spacer V17-V16
21	225P18	AY386697	16135	22502	Spacer V16-V15
22	225P18	AY386697	22794	32821	Spacer V15-V14
23	225P18	AY386697	33118	37738	Spacer V14-V13
24	225P18	AY386697	38044	44571	Spacer V13-V12
25	225P18	AY386697	44865	49447	Spacer V12-V11
26	225P18	AY386697	49745	56909	Spacer V11-V10
27	225P18	AY386697	57205	63678	Spacer V10-V9
28	225P18	AY386697	63977	71204	Spacer V9-V8
29	225P18	AY386697	71507	76261	Spacer V8-V7
30	225P18	AY386697	76560	79012	Spacer V7-V6
31	225P18	AY386697	79308	83467	Spacer V6-V5
32	225P18	AY386697	83768	88013	Spacer V5-V4
33	225P18	AY386697	88314	91233	Spacer V4-V3
34	225P18	AY386697	91531	95929	Spacer V3-V2
35	225P18	AY386697	96233	100963	Spacer V2-V1
36	225P18	AY386697	101262	133721	Spacer V1-D3

TABLE 1-continued

ID	BAC	Accession#	Start	Finish	Description
37	225P18	AY386697	133752	135212	Spacer D3-D1a
38	225P18	AY386697	135237	136922	Spacer D1a-D4
39	225P18	AY386697	136947	139446	Spacer D4-3' end
40	219D23	AY386695	8151	40612	Spacer V1-D3
41	219D23	AY386695	40643	42102	Spacer D3-D1a
42	219D23	AY386695	42127	43812	Spacer D4-D1b
43	219D23	AY386695	43837	48553	Spacer D1b-D6
44	219D23	AY386695	48577	48753	Spacer D6-D8
45	219D23	AY386695	48769	51181	Spacer D8-D2x
46	219D23	AY386695	51213	55826	Spacer D1c-Df
47	219D23	AY386695	55852	61112	Spacer Df-D1d
48	219D23	AY386695	61237	62445	Spacer D1d-D5
49	219D23	AY386695	62471	97024	Spacer D5-DQ52
50	219D23	AY386695	97036	97831	Spacer DQ52-J1
51	219D23	AY386695	97871	98090	Spacer J1-J2
52	219D23	AY386695	98140	98430	Spacer J2-J3
53	219D23	AY386695	98483	98642	Spacer J3-J4
54	219D23	AY386695	98690	98981	Spacer J4-J5
55	219D23	AY386695	99032	99550	Spacer J5-J6
56	219D23	AY386695	99604	106867	Spacer J6-IgM exon1
57	219D23	AY386695	107185	107290	Spacer IgM exon1-exon2
58	219D23	AY386695	107635	107863	Spacer IgM exon2-exon3
59	219D23	AY386695	108181	108268	Spacer IgM exon3-exon4
60	219D23	AY386695	108664	110499	Spacer IgM exon4-exonM1
61	219D23	AY386695	110615	110741	Spacer exonM1-exonM2
62	219D23	AY386695	108664	137302	Spacer IgM exon4-3' end
63	27N5	AY386696	5099	55582	Spacer IgM exon4-IgG exon1
64	27N5	AY386696	55867	56071	Spacer IgG exon1-exon2
65	27N5	AY386696	56104	56201	Spacer IgG exon2-exon3
66	27N5	AY386696	56531	56623	Spacer IgG exon3-exon4
67	27N5	AY386696	56946	58984	Spacer IgG exon4-exonM1
68	27N5	AY386696	56946	59205	Spacer IgG exon4-exonM2
69	27N5	AY386696	56946	69246	Spacer IgG exon4-IgE exon1
70	27N5	AY386696	69546	69719	Spacer IgE exon1-exon2
71	27N5	AY386696	70037	70132	Spacer IgE exon2-exon3
72	27N5	AY386696	70453	70532	Spacer IgE exon3-exon4
73	27N5	AY386696	70873	73061	Spacer IgE exon4-exonM1
74	27N5	AY386696	70873	73292	Spacer IgE exon4-exonM2
75	27N5	AY386696	70873	86059	Spacer IgE exon4-IgA4 exon1
76	27N5	AY386696	86362	86498	Spacer IgA4 exon1-exon2
77	27N5	AY386696	86876	87059	Spacer IgA4 exon2-exon3
78	27N5	AY386696	87450	89577	Spacer IgA4 exon3-exonM
79	27N5	AY386696	87450	103798	Spacer IgA4 exon3-IgA5b exon1
80	27N5	AY386696	104101	104231	Spacer IgA5b exon1-exon2
81	27N5	AY386696	104609	104787	Spacer IgA5b exon2-exon3
82	27N5	AY386696	105182	107227	Spacer IgA5b exon3-exonM
83	27N5	AY386696	105182	112077	Spacer IgA5b exon3-exonM
84	27N5	AY386696	105182	119223	Spacer IgA5b exon3-IgA1 exon1
85	27N5	AY386696	119511	119644	Spacer IgA1 exon1-exon2
86	27N5	AY386696	119984	120162	Spacer IgA1 exon2-exon3
87	27N5	AY386696	120557	122823	Spacer IgA1 exon3-exonM
88	27N5	AY386696	120557	127750	Spacer IgA1 exon3-exonM*
89	27N5	AY386696	120557	135838	Spacer IgA1 exon3-IgA2 exon1
90	27N5	AY386696	136138	136274	Spacer IgA2 exon1-exon2
91	27N5	AY386696	136652	136831	Spacer IgA2 exon2-exon3
92	27N5	AY386696	137229	139433	Spacer IgA2 exon3-exonM
93	27N5	AY386696	137229	146676	Spacer IgA2 exon3-3' end
94	Fos15B	AY386698	1	828	Spacer 5' start-IgA exonM
95	Fos15B	AY386698	1043	3596	Spacer IgA exonM-end contig1
96	Fos15B	AY386698	1	3596	Spacer 5' start-end contig 1
97	Fos15B	AY386698	7404	7541	Spacer IgA exon1-exon2
98	Fos15B	AY386698	7908	8086	Spacer IgA exon2-exon3
99	Fos15B	AY386698	8481	10538	Spacer IgA exon3-exonM
100	Fos15B	AY386698	8481	13140	Spacer IgA exon3-end contig2
101	Fos15B	AY386698	8481	15871	Spacer IgA exon3-1,2 hs 3' enh
102	Fos15B	AY386698	8481	21447	Spacer IgA exon3-4hs enh
103	Fos15B	AY386698	21484	33297	Spacer 4hs enh-end contig4
104	Fos15B	AY386698	16633	33297	Spacer 1,2hs 3' enh-end contig4
105	179L1	AY495827	1	124285	Spacer 5' end-enh
106	179L1	AY495827	125411	131350	Enhancer-C□
107	179L1	AY495827	131664	134637	Spacer C□-J5
108	179L1	AY495827	134684	134915	Spacer J5-J4
109	179L1	AY495827	134952	135196	Spacer J4-J3
110	179L1	AY495827	135241	135485	Spacer J3-J2
111	179L1	AY495827	135525	135863	Spacer J2-J1
112	179L1	AY495827	135897	155257	Spacer J1-V1
113	179L1	AY495827	155572	170621	Spacer V1-V2
114	179L1	AY495827	170936	173443	Spacer V2-V3

TABLE 1-continued

ID	BAC	Accession#	Start	Finish	Description
115	179L1	AY495827	173752	177227	Spacer V3-V4
116	179L1	AY495827	177536	185356	Spacer V4-V5
117	179L1	AY495827	185664	200758	Spacer V5-V6
118	179L1	AY495827	201064	203580	Spacer V6-V7
119	179L1	AY495827	203886	205144	Spacer V7-3' end
120	215M22	AY495826	1	12829	Spacer 5' end-V6
121	215M22	AY495826	13136	15653	Spacer V6-V7
122	215M22	AY495826	15957	22241	Spacer V7-V8
123	215M22	AY495826	22551	32876	Spacer V8-V9
124	215M22	AY495826	33188	38276	Spacer V9-V10
125	215M22	AY495826	38582	41476	Spacer V10-V11
126	215M22	AY495826	41780	47827	Spacer V11-V12
127	215M22	AY495826	48133	48547	Spacer V12-V13
128	215M22	AY495826	48841	51408	Spacer V13-V14
129	215M22	AY495826	51638	55438	Spacer V14-V15
130	215M22	AY495826	55745	67437	Spacer V15-V16
131	215M22	AY495826	67743	77805	Spacer V16-V17
132	215M22	AY495826	78120	80628	Spacer V17-V18
133	215M22	AY495826	80937	84009	Spacer V18-V19
134	215M22	AY495826	84315	87339	Spacer V19-V20
135	215M22	AY495826	87648	89399	Spacer V20-V21
136	215M22	AY495826	89711	95414	Spacer V21-V22
137	215M22	AY495826	95720	106650	Spacer V22-V23
138	215M22	AY495826	106956	110940	Spacer V23-V24
139	215M22	AY495826	111246	117877	Spacer V24-V25
140	215M22	AY495826	118183	122396	Spacer V25-V26
141	215M22	AY495826	122706	126496	Spacer V26-V27
142	215M22	AY495826	126802	133358	Spacer V27-V28
143	196O2	AY495828	37134	48826	Spacer V15-V16
144	196O2	AY495828	49032	59195	Spacer V16-V17
145	196O2	AY495828	115057	125885	Spacer V28-V29
146	196O2	AY495828	126195	130012	Spacer V29-V30
147	196O2	AY495828	130318	136966	Spacer V30-V31
148	196O2	AY495828	137272	144512	Spacer V31-V32
149	196O2	AY495828	144819	148617	Spacer V32-V33
150	196O2	AY495828	148923	155402	Spacer V33-V34
151	196O2	AY495828	155714	171415	Spacer V34-V35
152	196O2	AY495828	171572	177676	Spacer V35-V36
153	196O2	AY495828	177979	178083	Spacer V36-3' end
154	CLC*	NA	1	443	Spacer 5' end-pV28**
155	CLC*	NA	486	1203	Spacer pV28-pV27**
156	CLC*	NA	1528	1635	Spacer pV27-pV26**
157	CLC*	NA	1818	2242	Spacer pV26-pV25**
158	CLC*	NA	2585	2676	Spacer pV25-pV24**
159	CLC*	NA	2781	3327	Spacer pV24-pV23**
160	CLC*	NA	3464	3659	Spacer pV23-pV22**
161	CLC*	NA	3985	4241	Spacer pV22-pV21**
162	CLC*	NA	4578	4994	Spacer pV21-pV20**
163	CLC*	NA	5366	5425	Spacer pV20-pV19**
164	CLC*	NA	5749	5842	Spacer pV19-pV18**
165	CLC*	NA	6034	7043	Spacer pV18-pV17**
166	CLC*	NA	7266	7493	Spacer pV17-pV16**
167	CLC*	NA	7625	7625	Spacer pV16-pV15**
168	CLC*	NA	7988	8758	Spacer pV15-pV14**
169	CLC*	NA	9100	9410	Spacer pV14-pV13**
170	CLC*	NA	9787	10057	Spacer pV13-pV12**
171	CLC*	NA	10441	11022	Spacer pV12-pV11**
172	CLC*	NA	11380	11911	Spacer pV11-pV10**
173	CLC*	NA	12162	12349	Spacer pV10-pV9**
174	CLC*	NA	12691	13357	Spacer pV9-pV8**
175	CLC*	NA	13708	13882	Spacer pV8-pV7**
176	CLC*	NA	14229	14406	Spacer pV7-pV6**
177	CLC*	NA	14599	15338	Spacer pV6-pV5**
178	CLC*	NA	15613	16578	Spacer pV5-pV4**
179	CLC*	NA	16916	18219	Spacer pV4-pV3**
180	CLC*	NA	18439	18879	Spacer pV3-pV2**
181	CLC*	NA	19248	19343	Spacer pV2-pV1**
182	CLC*	NA	19609	22208	Spacer pV1-V**
183	CLC*	NA	22506	24313	Spacer V-J
184	CLC*	NA	24350	26088	Spacer J-C□
185	CLC*	NA	26402	36259	Spacer C-3' end

*CLC—Chicken light chain locus SEQ ID 184, FIG. 9

**pV—pseudo V gene (not functional)

Comments

BAC sequences submitted to GenBank were modified by deletion of vector sequences at the 5' and 3' end as follows:

BAC	Accession#	Removed from 5' end	Removed from 3' end
38A2	AY386694	1-125	1281285-128225
219D23	AY386695	1-54	137357-137389

-continued

BAC	Accession#	Removed from 5' end	Removed from 3' end
5 Fos15B*	AY386698	1-97	33395-33427
179L1	AY495827	0	205145-205968
196O2	AY495828	1-32	178117-178171

*In addition contigs in GenBank are separated by 50 nt. In the Fos15B sequence submitted with the provisional application contigs were separated by 10 nt.

TABLE 2

ID	Region	Sequence
193	V _H 1	5' CGCGGATCCGAGACTGGGCTGCGCTG3'
194	V _H 1	5' CGCAAGCTTGAATAGGTGGCCGTGTC3'
195	J _H	5' CGCGGATCCAGGCACCCTGGTCACCG3'
196	J _H	5' CGCAAGCTTGTGACCAGGGTGCCCTG3'
197	C _γ	5' CGCGGATCCCTGGAGCCGAAGGTCTAC3'
198	C _γ	5' CGCAAGCTTGAGATGGACTTCTGCGTG3'
199	3' Enh	5' CGCGGATCCCAGAGTGGTCTGTGACA3'
200	3' Enh	5' CGCAAGCTTACAGGCGCATGCAAATGC3'
201	V _K	5' CGCGGATCCGAGGCACAGTCACCATC3'
202	V _K	5' CGCAAGCTTACAGTAGTAAGTGGCAGC3'
203	J _K	5' CGCGGATCCGAGGGACCGAGGTGGT3'
204	J _K	5' CGCAAGCTTACCATGGTCCCTGAGCC3'
205	C _κ	5' CGCGGATCCCCTCAGGTGATCCAGTTG3'
206	C _κ	5' CGCAAGCTTCTATTGAAGCTCTGGACG3'
207	K 3' Enh	5' CGCGGATCCGTGACTGGCCCAAGAAG3'
208	K 3' Enh	5' CGCAAGCTTATACAACCTTGGCCAGG3'
209	C _κ	5' AAACAGCTTTTACACCTCCCCTTTCTCTTTGCTCCCC TGGGCCCTCAGGGAGTGCATCCGCCCAACCCTTTTCC3'
210	C _κ	5' CAGGGTTAGTTTGCATGCACACACACAGCGCCTGGTC ACCCAGAGGGTTCAGTAGCAGGTGCCAGCTGTGTCCGACATG3'
211	C _κ	5' GGTCAGGGTCTCCAGGGCAGGGTACATTGTGCCCC TTCTTTGACGCTCCACCAAGGGCCCATCGGTC3'
212	C _γ	5' CACAGCTGCGGCGTGGGGGGAGGGAGAGGGCAGCTCG CCGGCACAGCGCTCATTTACCCGGAGACAGGGAGAGGCTCTTC3'
213	J _H	5' GTGTTATAAAGGGAGACTGAGGGGGCAGAGGCTGTGCTA CTGGTACCTGGCTGAATACTTCCAGCACTGGGGCCAGG3'
214	J _H	5' GGCCACAGAAAAGAGGAGAGAATGAAGCCCCGGAGAG GCCGTTCTACCTGAGGAGACGGTGACCGTGGTCCCT TG-3'
215	Genta	5' CCAGGCCGGCCTGGAGTTGTAGATCCTCTACG3'
216	Genta	5' CCAGGCGCGCCAAGATGCGTGATCTGATCC3'
217	Linker	5' GGCCGCGGCCGGCCATCGATGGCGCGCCTTCGAAACCGTA3'
218	Linker	5' AGCTTACGCTTTTCAAGGCGCGCCATCGATGGCCGGCCG3'
219	pBB11.1	5' ATTCCCAAGCTTTTAATTAAGACGTACGCTTCTTAGCTCCTG3'
220	pBB11.1	5' ATTCGCGGATCCACGCGTTTCGTTCCCAAAGGCGCGCTAGCG ATGAGCTCGGAC3'

TABLE 2-continued

ID	Region	Sequence
221	Neo	5' GCAGGCATGCAAAGCTTATTACACCAGTGTTCAGTAAGCG3'
222	Neo	5' GGTACCCGGGGATCCTCAGAAGAAGTTCGTCAAGAAGGCG3'
223	pBB11.2	5' AAATTCCTTAATTAAGACGTCAGCTTCCTTAGCTCCTG3'
224	pBB11.2	5' GAAACCGGGGACGCGTTACCGTTCGTATAATGTATGCTATACGAA GTTATGCGGCCGCTAGCGATGAGCTCGGAC3'
225	CA	5' TTCTCTGTTTTTGTCCGTGGAATGAACAATGGAAGTCCGAGCTCA TCGCTAAGGGCACCAATAACTGC3'
226	CA	5' CACAGGAGAGAAACAGGACCTAGAGGATGAGGAAGTCCCTGTAG GCTTCTACCGTTCGTATAATGTATGCTATACGAAGTATTACCTGT GACGGAAGATC-3'
227	V _H 3-9	5' ATAGAGAGATTGAGTGTG3'
228	V _H 3-9	5' TCCTGTCTTCCTGCAG3'
229	V _H 3-11	5' AGAGACATTGAGTGGAC3'
230	V _H 3-11	5' AGGGAGGTTTGTGTC3'
231	V _H 3-13	5' ACTAGAGATATTGAGTGTG3'
232	V _H 3-13	5' AGGCATTCTGCAGGG3'
233	V _H 3-15	5' ACTAGAGAGATTAAGTGTG3'
234	V _H 3-15	5' TCACACTGACCTCCC3'
235	V _H 3-20	5' TCATGGATCAATAGAGATG3'
236	V _H 3-20	5' TGCAGGGACGTTTGTG3'
237	V _H 3-23	5' AGAAAAATTGAGTGTGAA3'
238	V _H 3-23	5' GTGTCTGGGCTCACAA3'
239	V _H 3-30	5' AGAGAGACTGAGTGTG3'
240	V _H 3-30	5' TGCAGGGAGGTTTGTG3'
241	V _H 3-43	5' TGAGTGTGAGTGAACATG3'
242	V _H 3-43	5' ACCAGCTCTTAACCTTC3'
243	V _H 3-64	5' TGAGTGTGAGTGGAC3'
244	V _H 3-64	5' TGACGCTGATCAGTG3'
245	V _H 3-66	5' TCTGACCAATGTCTCTG3'
246	V _H 3-66	5' AGGTTTGTGTCTGGGC3'
247	V _H 3-72	5' ACAAGGTGATTTATGGAG3'
248	V _H 3-72	5' AGGTTTGTGTCCGGG3'
249	V _H 3-9	5' TTGGCGCGCC TGTCGTCTGTGTTTGCAG GTGTCC3'
250	V _H 3-9	5' TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCTCACTGTGTCTCTTGCAC3'
251	V _H 3-11	5' TTGGCGCGCC TGTCGTCTGTGTTTGCAG GTGTCC3
252	V _H 3-11	5' TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCTCACTGTGTCTCTCG3'
253	V _H 3-13	5' TTGGCGCGCCTGTCTGTGTTTGCAGGTGTCC3'
254	V _H 3-13	5' TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCTCACTGTGTCTCTTGC3'
255	V _H 3-15	5' TTGGCGCGCCTGTCTGTGTTTGCAGGTGTCC3'

TABLE 2-continued

ID	Region	Sequence
256	V _H 3-15	5' TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCCTCACTGTGTCTGTGG3'
257	V _H 3-20	5' TTGGCGCGCC TGTCGTCTGTGTTTGCAGGTGTC3'
258	V _H 3-20	5' TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTC AGCCTGAGGGCCCCCTCACTGTGTCTCTC3'
259	V _H 3-23	5' TTGGCGCGCCTGTCTGTGTGTTTGCAG GTGTCCAGTGTG3'
260	V _H 3-23	5' TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCAGCCTGA GGGCCCTCACTGTGTCTTTC3'
261	V _H 3-30	5' TTGGCGCGCCTGTCTGTGTGTTTGCAGGTGTCCAGTGTG3'
262	V _H 3-30	5' TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCAGCCTG AGGGCCCCCTCACTGTGTCTTTCG3'
263	V _H 3-43	5' TTGGCGCGCCTGTCTGTGTGTTTGCAGGTGTCC3'
264	V _H 3-43	5' TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCCTCACTGTGTCTCTTTCGAC3'
265	V _H 3-64	5' TTGGCGCGCCTGTCTGTGTGTTTGCAGGTGTCC3'
266	V _H 3-64	5' TTGCACGCGTGCAGGCAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCCTCACTGTGTCTCTCGCAC3'
267	V _H 3-66	5' TTGGCGCGCCTGTCTGTGTGTTTGCAGGTGTCC3'
268	V _H 3-66	5' TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCCTCACTGTGTCTCCG3'
269	V _H 3-72	5' TTGGCGCGCCTGTCTGTGTGTTTGCAG GTTTC33'
270	V _H 3-72	5' TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCCTCACTGTGTCTCTAGCAC3'
271	V1-2	5' TTGGCGCGCCAGGGGAGTGC GGCTCCAC3'
272	V1-2	5' TTGCACGCGT TGGTCAGGACTGTCACTCAC3'
273	V2-3	5' TTGGCGCGCCAGGGGCGCGGGCTCCAC3'
274	V2-3	5' TTGCACGCGTTGATCACGAACTGTCACTCACACTCTC3'
275	V3-4	5' TTGGCGCGCCAGGGGCGCGGGCTCCAC3'
276	V3-4	5' TTGCACGCGTTCTGTGGTCTCTTCTTCTCTTGCTATAAC3'
277	V4-5	5' TTGGCGCGCCAGGGGAGTGC GGCTCCAC3'
278	V4-5	5' TTGCACGCGTTGGTCAAGACTGTCACTCAC3
279	V5-6	5' TTGGCGCGCCAGGGACGCACGGCTCCAC3'
280	V5-6	5' TTGCACGCGTTGGTCAGGAAGCTGTCACTCAC3'
281	V6-7	5' TTGGCGCGCCAGGGATGCGGGCTCCAG3'
282	V6-7	5' TTGCACGCGTTGGTCAGGACTGTCACTGACAC3'
283	V7-8	5' TT GGCGCGCCAGGGGAGTGC GGCTCCAC3'
284	V7-8	5' TTGCACGCGTTGGTCAGGAAGCTGTCACTCACTCTC3'
285	V21-22	5' TT GGCGCGCCGGGGCCCGGGCTCCAC3'
286	V21-22	5' TTGCACGCGTTGGTCAGGAAGCTGTCACTCAC3'
287	V22-23	5' TTGGCGCGCCAGGGACGTGAGGCTCTAC3'
288	V22-23	5' TTGCACGCGTTGGTCAGGGCACTGTCACTCAC3'
289	Linker	5' GGCCGCGCCGGCCATCGATGGCGCGCC TTCGAAACGCGTA3'
290	Linker	3' CGCCGGCCGGTAGCTACCGCGGGAAGCTT TGCGCATTCGA5'

TABLE 2-continued

ID	Region	Sequence
291	Linker	5' CCG CCG GCC ATC GAT GGC GCG CCT TCG AAA CGC GTG GTA C3'
292	Linker	3' TCG AGC CGG CCG GTA GCT ACC GCG CGG AAG CTT TGC GCA C5'
293	Genta	5' CCAGGCCGGCCTGGAGTTGTAGATCCTCTACG3'
294	Genta	5' CCAGGCGGCCAAGATGCGTGATCTGATCC-3'
295	Neo	5' CCAGGCCGGCCATTACACCAGTGTGAGTAAGCG3'
296	Neo	5' CCAGGCGCGCCTCAGAAGAACTCGTCAAGAAGGCG3'
297	Linker	5' GAT CCC GCC GGC CAT CGA TGG CGC GCC TTC GAA ACG CGT TAG GGA TAA CAG GGT AAT A3'
298	Linker	3' GCC GGC CGG TAG CTA CCG CGC GGA AGC TTT GCG CAA TCC CTA TTG TC CCA TTA TCGA5'
299	Neo	5' ATCTGCACTCAGTGCCTTTGAGCGCCCCCTGGTAGAGCCG CGCGACCCT GGCGCGCC ATTACACCAGTGTGAGTAAGCG 3'
300	Neo	5' AAATGACCAGTCTGACAGCCGCGGACACGGCCACCTATTTT TGTCGAGA GGCCGGCC TCAGAAGAACTCGTCAAGAAGGCG3'
301	Ck Km3	5' GATGTCCACTGGTACCTAAGCCTCGCCCTCTGTGCTTCTTCCCTC CTCAGGAAGTGTGGCTGCACCATCTGTCTTC3'
302	Ck Km3	5' GAGGCTGGGCCTCAGGGTCGCTGGCGGTGCCCTGGCAGGCGTC TCGCTCTAACACTCTCCCCGTTGAAGCTCTTTGTG3
303	Neo	5' CTTTCTCTGTCTTCTGTGCGACGGTTACGCCGCTCCATGAGCTT ATCGTAACTATAACGGTCTAAGGTAGCGATGGACAGCAAGCGAA CCGGA3'
304	Neo	5' GGACCAGTTTACAATCCACCTGCCATCTAAGAAAGCTGGTCTCA TCGTGTCAGAAGAACTCGTCAAGAAG3'
305	Zeo	5' CCCCCCGCCACTTCTTCTGTTTCGTTTAAAGTTCTACTGAC ATACTAGGATAACAGGGTAATAACGTTTACAATTCGCCTGATG3
306	Zeo	5' AGTGGGTAGGCCTCGCGCCGCGCTGGCCGTCGACATTTAGGTGA CACTATAGAAGGATCCTAGCACGTGTGAGTCTGCT3'
307	Genta	5' TTACGCCAAGCTATTTAGGTGACACTATAGAATACTCAAGCTTTG ATGCTAACTATAACGGTCTAAGGTAGCGATGAAGGCAGAACCC AGTTG3'
308	Genta	5' GCGGAATTCTATGTCTAGTGGAGGGTGAAGCTGGTGATTATAGA GTGAAAATTACCTGTTATCCCTATCGGCTTGAACGAATTGTTAG3'
309	VJ	5' CATAAATATACTGTCTTCCAGGATCTTAGAGCTCACCTAAGGAAA CAAGAGTTCATTTGAAGTTTTTAAAGTG3'
310	VJ	5' ACTCCAGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGAAT AGGAACTTCCTTTGATCTCCACCTTGGTC3'
311	Genta	5' GAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAAC TTCTGGAGTTGTAGATCCTCTACG3'
312	Genta	5' AAAACAAACCAATCAGGCAGAAACGGTGAGGAATCAGTGAAAC GGCCACTTACGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGG AATAGGAACTTCAAGATGCGTGATCTGATCC3'
313	FRT	5' TTATGCTGCATCCAGTTTG3'
314	FRT	5' AAAACAAACCAATCAGGCAG3'
315	FRT	5' TGTGACATCCAGATGAC3'
316	FRT	5' AAAACAAACCAATCAGGCAG3'
317	Genta	5' GGACCAGTTTACAATCCACCTGCCATCTAAGAAAGCTGGTCTCA TCGTGGTGCCAGGGCGTGCCTTGGGCTGGGGCGCGATAACTTCG TATAGCATACATTATACGAAGTTATCGATCGTGGAGTTGTAGATCC TCTACG3'

TABLE 2-continued

ID	Region	Sequence
318	Genta	5' TTACGCCAAGCTATTTAGGTGACACTATAGAATACTCAAGCTTTG ATTGCAAGATGCGTGATCTGATCCT3'
319	Linker	5' CGGGATCCGCGCGTACGGAAGTTCCTATACCTTTGAAGAATAGG AACTTCGGAATAGGAACATTCATTACACCAGTGTCAAGTAAGCG3'
320	Linker	5' GGAAGCTTCGCGCGATCGCCGCTTTCGCAAAGGCGCGCCTCAG AAGAACTCGTCAAGAAGGCG3'
321	Genta	5' GGCGGCCGCTGGCCGTCGACATTTAGGTGACACTATAGAAGGA TCCGCGTGGAGTTGTAGATCCTCTACG3'
322	Genta	5' AACTCAGTAAGGAAAAGGACTGGGAAAGTGCACCTTACATTTGAT CTCCAGGCGCGCAAGATGCGTGATCTGATCC3'
323	Neo	5' GGACCAGTTTACAATCCCACCTGCCATCTAAGAAAGCTGGTCTCA TCGTGGTGCCAGGGCGTGCCCTTGGGCTGGGGGCGCGGAAGTTCTT ATTCCGAAGTTCTTATTTCTCAAAGGTATAGGAACTTCCGTACGA TTACACCAGTGTCAAGTAAGCG3'
324	Neo	5' GGACTGATGGGAAAATAGAGGAGAAAATTGACCAGAGGAAGTG CAGATGGTCAGAAGAACTCGTCAAGAAGGCG3'
325	RSS	5' AACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTA CCCCTTCCACAGTGATACAAGCCC3'
326	RSS	5' TGCCGGCCACGATGCGTCCGGCGTAGAGGATCTACAACCTCCAGG CGCGCTGGTTCATGTCAAGTGTCTGCTGC3'
327	Genta	5' CTCCTTTCCTCCTCCTTGGTGGCAGCAGCACTGACATGACCAGGC GCGCC TGGAGTTGTAGATCCTCTACG 3'
328	Genta	5' TGTAATACGACTCACTATAGGGCGAATTCGAGCTCGGTACCCGGG GATCCCGTACGAAGATGCGTGATCTGATCC3'
329	Kana	5' GGCGGCCGCTGGCCGTCGACATTTAGGTGACACTATAGAAGGA TCCGCGACCCTGTTATCCCTAGATTTAAATGATATCGG 3'
330	Kana	5' AACTTTCTCCTACAGATCCCAGATAACCATGAATTTATTACACCA TCTTGGGCGCGCCGAAGTTCTTATACCTTTCTAGAGAATAGGAACTT CGGAATAGGAACTTCAGTTGGTGATTTTGAACTTTGTCTTGGCC3'
331	Amp	5' GGACCAGTTTACAATCCCACCTGCCATCTAAGAAAGCTGGTCTCA TCGTGGTGCCAGGGCGTGCCCTTGGGCTGGGGGCGCGGCGATCGCG AAGTTCCTATTCCGAAGTTCTTATTTCAAAGGTATAGGAACTTC TACGGGTCTGACGCTCAG 3'
332	Amp	5' GAATTCAGAGCTCAATGAGTTGCCTTGTTCAGAGCTCTATTTTCA CTTGACGTACGACAGACAAGCTGTGACCGTC3'
333	J Region	5' GAGTTAGGCCTCAGAGCTGAGGCAGGGCTCGGTTCCCTTGGGTG AGAAGGGTTTCTGTTTCAGCAAGAC3'
334	J Region	5' TGGCCAATTAGAGCAAAATTTAGACAGTAATAGGAAAAAGGTA CTTACGTTTAAATCTCCAGTCTGTC3'
335	O2	5' TCAGTACTGACTGGAAC3'
336	O2	5' CCAATGACTTTCAAACC3'
337	L8	5' CCGTACAGCCTGGCTC3'
338	L8	5' AACACCATCAGAGTGTGC
339	L4	5' ATGATTAATTGTGTGGACC3'
340	L4	5' AGGTGATCTCATATCCTC3'
341	A30	5' CTCAGTACTGCTTTACTG3'
342	A30	5' TGACTTCATGTCCCCTTC3'
343	L11	5' ACATGATTAATTGTGTGGACC3'
344	L11	5' GGTGCAGAGGTGACTTCG3'
345	L1	5' CTCAGTACTGCTTTACTATTC3'

TABLE 2-continued

ID	Region	Sequence
346	L1	5' GAGGAACACTCTCAGCTG3'
347	L5	5' CAGGGAACCTTCTCTTACAG3'
348	L5	5' GAATTAGGGTGCAGAGGC3'
349	L15	5' TACTATTCAGGGAAATTC3'
350	L15	5' TGTCTGTGAAGTTGGTG3'
351	O8	5' TGGCTCTTGATGGAAGC3'
352	O8	5' ACTTCAAAGTGTGACTGC3'
353	L19	5' AGGGAACCTTCTCTTACAGC3'
354	L19	5' AATTAGGGTGCAGAGGCG3'
355	L12	5' GAAGTCTTCTATAATATGATC3'
356	L12	5' TGGCTGCATCTGAGGACC3'
357	A20	5' GCCACTAATGCCTGGCAC3'
358	A20	5' CTGCTGTCAGCAGAGGGC3'
359	O4	5' CTTCTTATAACATGATGG3'
360	O4	5' AAACGCTCTGAGCAGC3'
361	L14	5' CTCAGTACTGCTTTACTG3'
362	L14	5' GAGGAACAATCTCAGCCG3'
363	L23	5' AGCCAGGCTGTACGGAAC3'
364	L23	5' CCCAGCCTCACACATCTC3'
365	L9	5' TGGCCCTTCAGGGAAG3'
366	L9	5' ACCATCAGAGTGTGGTTG3'
367	A4	5' CCAGTGTAGCCATTAATG3'
368	A4	5' TACCAAAACCTCCAGGG3'
369	L24	5' GGGAAATTCTCTTACTAC3'
370	L24	5' CCCCCTCTACCAATAC3'
371	O6	5' CCATTCAGGGAAGTCTTC3'
372	O6	5' TGAGTCTGAGAAGTGTG3'
373	L22	5' GGAATTTCTTAGCCAC3'
374	L22	5' ATGTTCAGGCTTGTAACC3'
375	A9	5' TCATCTTACAAATAGTTG3'
376	A9	5' TCTGACCATTCCCTGC3'
377	A25	5' GGGAAATCATCTTATAAATAG3'
378	A25	5' TGCAGATGAGACTTCTGG3'
379	A15	5' ATTCAGGAAAGTCCTCTC3'
380	A15	5' CAGTGACCTTCAGAGTG3'
381	O9	5' ATTCAGGAAAGTCCTCTC3'
382	O9	5' CAGTGACCTTCAGAGTG3'
383	O2	5' TTGGCGCGCCTTCTGTTCCCTTCTCAGGTGCCAGATGTGACATC
384	O2	5' CGCACGCGTGTGTTGATCTCCACCTTGGTCCCTCCGCCGAAAGTGA GAGGGTACTGTAACCTGTGTTG3'

TABLE 2-continued

ID	Region	Sequence
385	L8	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGACATC
386	L8	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATTAAG3'
387	L4	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGCCATC
388	L4	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATTAAC3'
389	A30 7	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGGTGTGACATC3'
390	A30	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATTATGC3'
391	L11	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAG GTGCCAGATGTGCCATC3'
392	L11	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAATTGTAATC3'
393	L1	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGACATC3'
394	L1	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATTATAC3'
395	L5	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAG GTCCAGATGCGACATC3'
396	L5	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGAAACTGTTAG3'
397	L15	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGACATC
398	L15	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATTATAC3'
399	O8	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGACATC
400	O8	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGAGATTATCATAC3'
401	L19	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAG GTCCAGATGCGACATC3'
402	L19	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGAAACTGTTAG3'
403	L12	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAG GTGCCAAATGTGACATC3'
404	L12	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGAATAACTATTATAC3'
405	A20	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAG ATACCAGATGTGACATCC3'
406	A20	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGCACTGTTATAC3'
407	O4	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAGGT GCCAGATGTGACATC3'
408	O4	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGCATTGTAAG3'
409	L14	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAG GTGCCAGATGTAACATCC3'
410	L14	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATTATGC3'
411	L23	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAG GTGCCAGATGTGCCATC3'

TABLE 2-continued

ID	Region	Sequence
412	L23	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTACTATAATAC3'
413	L9	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAG GTGCCAGATGTGCCATC3'
414	L9	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATAATAC3'
415	A4	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAG ATACCAGATGTGACATCC3'
416	A4	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGCACTGTTATAC3'
417	L24	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGTCATC3'
418	L24	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGAACTATAATAC3'
419	O6	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAG GGACCAGAAGTGACATC3'
420	O6	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAATTTTATAC3'
421	L22	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGTCAGATTTGACATCC 3'
422	L22	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTGAAGTC3'
423	A9	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAG GAGTCAGATGTGATTTCC3'
424	A9	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GATGGCTGCTGTAAG3'
425	A25	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAG GAGTCAGATGTGATTTCC3'
426	A25	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GATGGCTGCTGTAAG3'
427	A15	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATAT GACATGC3'
428	A15	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTCACTTTTATAC3'
429	O9	5' TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGC CAGATATGACATGC3'
430	O9	5' CGCACGCGTCCTGGGGGGTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTCACTTTTATAC3'
431	V7-8	5' TTGGCGCGCC GGAGGAAACAGAAACACAG3'
432	V7-8	5' CGCACGCGT CAGCTGCTCGTCCTGGG3'
433	V1-10	5' TTGGCGCGCC GGAGGGAAACAGAAACAC3'
434	V1-10	5' CGCACGCGTAGCTGCTCCTCCTGGG3'
435	V15-14	5' TTGGCGCGCC GAAGGGAAACAGAAACACAG3'
436	V15-14	5' CGCACGCGTAGCTGCTCCTCCTGGG3'
437	V18-17	5' TTGGCGCGCC GAGGGAAACAGAAACAC3'
438	V18-17	5' CGCACGCGTCAGCTGCTGCTCCTGGG3'
439	V19-18	5' TTGGCGCGCC GAAGGGAAACAGAAACACAG3'
440	V19-18	5' CGCACGCGT AGCTGCTCCTCCTGGG3'
441	V20-19	5' TTGGCGCGCC GAGGAGGAAACAGAAACAC3'

TABLE 2-continued

ID	Region	Sequence
442	V20-19	5' CGCACGCGTCAGCTGCCCTCCTGGG3'
443	V21-20	5' TTGGCGCGCC GGAGGAAACAGAAACACAG3'
444	V21-20	5' CGCACGCGTCCCTAGCTGCTCCTGGG3'
445	V24-23	5' TTGGCGCGCC GGAGGAAACAGACACAC3'
446	V24-23	5' CGCACGCGTCAGCTGCTCCTCCTGGG3'
447	V26-25	5' TTGGCGCGCC GAAGGAAAGAGAAACACAG3'
448	V26-25	5' CGCACGCGTAGCTGCTCCTCCTGGG3'
449	V27-26	5' TTGGCGCGCC GGAGGAAACAGAAACAC3'
450	V27-26	5' CGCACGCGTCCCAGCTGCTCCTGGG3'
451	Genta	5' AGCTCGGTACCCGGGATCCTCTAGAGTCGACCTGCAGGC ATGCAAGCTTGGCCGGCCTGGAGTTGTAGATCCTCTACG3'
452	Genta	5' AAAACAAACCAATCAGGCAGAAACGGTGAGGAATCAGT GAAACGGCCACTTACGGCGCGCAAGATGCGTGATCTGATCC3'
453	Hygro	5' CGTTGGACCAGTTTACAATCCACCTGCCATCTAAGAAAGC TGGTCTCATATAACTTCGTATAATGTATGCTATACGAACGGTA ACGCGTGAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGT ATAGGAACCTTCTCAGAGCAGATTGTACTG3'
454	Hygro	5' GGACAGCAAGCGAACCGGAATTGCCAGCTGGGGCGCCCTCT GGTAAGGTTAAGATGCGTGATCTGATCC3'
455		5' ACTGCACCTCAGCGTCCCCCTGCCATGTCAGGGCCGATGAA GGGCACAGCGTACGATTACACCAGTGTCAGTAAGCG 3'
456		5' TGTAATACGACTCACTATAGGGCGAATTGAGCTCGGTACCCG GGGATCCTGCGATCGCTCAGAAGAACTCGTCAAGAAGGCG 3'
457	J _H	5' GTGTTATAAAGGGAGACTGAGGGAGGCAGAGGCTGTGCTA CTGGTACCTGGCTGAATACTTCCAGCACTGGGGCCAGG3'
458	J _H	5' GGCCACAGAAAAGAGGAGAGAATGAAGGCCCGGAGAGG CCGTTCCCTACCTGAGGAGACGGTGACCGTGGTCCCTTG3'
459	Spacer	5' AACAACTCAGGGCTGAGGACACC3'
460	Spacer	5' CTGCCCGTTGTCCCTCGAGATGGTGGCACGGCC3'

SEQUENCE LISTING

The patent contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (<http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US08410333B2>). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

What is claimed is:

1. A transgenic rabbit, comprising a humanized immunoglobulin locus comprising multiple Ig gene segments wherein

(a) at least one of said gene segments is a human Ig gene segment comprising two or more identical or different units consisting of, from 5' to 3' direction, a 5' nucleotide sequence, a human immunoglobulin heavy or light chain V gene segment, and a 3' nucleotide sequence, wherein said 5' nucleotide sequence and said 3' nucleotide sequence have the nucleotide sequence of SEQ ID NO 35;

(b) said gene segments are juxtaposed in an unrearranged, partially rearranged or fully rearranged configuration, and

(c) said humanized Ig locus is capable of undergoing gene rearrangement, if necessary, and gene conversion and/or hypermutation, and producing a repertoire of humanized immunoglobulins in said transgenic rabbit.

2. The transgenic rabbit of claim 1 which preserves an essentially intact endogenous regulatory and antibody production machinery.

* * * * *